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Study on the Locally Available Aquatic Macrophytes as Fish Feed For Rural Aquaculture Purposes in South America

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To my beloved mother Judith

It is commonly known that aquaculture needs to increase further its net contribution to the total world fish supplies. However, at present almost all farming operations, based on the use of fish feed, are highly dependent on available fishery resources for the production of fish meal, thus becoming a reducing activity rather than an activity producing fishery resources. The quantity of inputs of dietary fishery resources (principally in the form of fishmeal and fish oil) exceeds considerably the outputs in terms of farmed fishery products. This is the reason why nutritionists have been searching worldwide for effective fish meal substitutes, and several attempts have been made to partially or totally replace fish meal with less expensive protein sources.

If the aquaculture growth potential is to be realized and maintained, then considerable quantities of nutrient inputs in the form of fertilizers, supplementary feeds or complete compound aquafeeds will have to be available on a sustainable basis. It is evident on a long-term that the small producers will be unable to depend on commercial aquafeeds based traditionally on fish meal, due to its increased price. Therefore, there is a need to provide and assure small-scale farmers an alternative fish feed wherever possible based on the use of non-food grade locally feed resources, which is available in rural areas, which is low-cost and which is suitable for the proper growth and maintenance of native fish. Thus, plant source proteins are a logical choice for replacing fish meal in diets for herbivorous and omnivorous species, while animal protein sources are clearly preferred alternatives to fish meal in carnivorous species.

Aquatic plants are considered important nutritional sources for herbivorous-omnivorous fish and in many cases they could replace up to 25% of formulated diets and up to 50% of commercial feeds (35% protein) without adverse effects on fish growth and body composition. In many parts of the world, aquatic plants are widely distributed and can be considered as plague. This occurs particularly in tropical countries as Colombia due to abundant sunlight and favorable water temperature.

However, the use of this resource has some limitations. Chemical composition of aquatic plants is highly affected by the aquatic environment in which they grow. There have been few controlled laboratory studies evaluating the nutritional characteristics of the available aquatic plants in Colombia. Likewise, the use of plant-derived materials as fish feed ingredient is limited by the presence of wide variety of constituents or antinutrients that affect the normal fish growth negatively; so that plants may be processed to reduce the effects of these compounds. There is little or limited information on the potential or processing of the local

aquatic plants that can be used to optimize their inclusion in diets for native fish species in Colombia. Considering the aspects mentioned above, the general objective of this study was to assess the nutritional potential of aquatic plants available in rural Colombia treated by sun drying and by fermentation as well as to evaluate the effect of their use as fish feed on the growth performance of common cultured tropical fish (*Piaractus brachypomus* and *Oreochromis niloticus*) fed low fishmeal diets (3%) and until 25% of aquatic plants.

In **Chapter 1** the nutritional characteristics of *Lemna minor*, *Spirodela polyrhiza*, *Azolla filiculoides* and *Eichhornia crassipens* and its potential for fermentation were evaluated. Aquatic macrophytes were harvested as wild or uncultivated material from water bodies in rural areas from Colombia. Analytical methods for nutrient content and antinutrients concentration were carried out following standard procedures. Fermentation characteristics of the aquatic macrophytes were evaluated according to the Deutsche Landwirtschafts-Gesellschaft (DLG) method which is used as a key to the evaluation of the fermenting quality of forage ensilage on basis of the chemical investigation. The results showed that the locally available species have an acceptable protein content from 98 to 243g kg^{-1}, a raw lipid content from 13 to 39 g kg^{-1} and a relatively high fibre (> 128 g kg^{-1}) and ash content (>145 g kg^{-1}). The content of ash and fibre for the tested plants in this study was however comparable with values reported in the literature for several aquatic plants. The profile of amino acids was similar in all of the plants from 5.3 to 6.3 g lysine per 100 g of protein and from 1.7 to 2.0 g methionine per 100 g of protein and the experimental diets containing the aquatic plants met the nutritional requirements of the studied fish.
Aquatic plants showed a high buffer capacity from 70 to 90 g kg^{-1} lactic acid, a low content of soluble sugar (<10 g kg^{-1}) and a high moisture content (>90%), which hinders the fermentation. However, the reduction in the moisture content (<50%) and the use of additives – a bacterial inoculant (source for Lactobacillus) and molasses (source for soluble carbohydrates) – resulted in silages of a very good quality (>90 points of DLG). The fermentation process reduced significantly the fibre content in *Lemna minor, Spirodela polyrhiza* and *Azolla filiculoides* and the concentration of antinutrients was also lower in the fermented material than in the fresh plant material. The concentrations of trypsin inhibitor (<1.4 mg TI g^{-1}), oxalates (1.9 g kg^{-1}), phytates (2.0 g kg^{-1} phytic acid) and soluble tannins and condensates (no detectable) in the fermented aquatic plants did not exceed the tolerable limits reported for fish.
In **Chapter 2** the apparent digestibility coefficients (ADC) of protein and energy of sundried and fermented *Lemna minor, Spirodela polyrhiza* and *Azolla filiculoides* was determined for

the fish species *Piaractus brachypomus*. Determination of ADC was carried out using a semi-purified diet as reference. In all the cases 30% of the reference diet was replaced with the sundried aquatic macrophytes and the aquatic macrophytes treated by lactic acid fermentation. Six experimental diets and one reference diet were offered to triplicated groups of fish during a period of 30 days. After that, faecal samples were collected with a maximal interval of one hour until the required amount for analyses was obtained. The Experiment was conducted in a modified Guelph system. The ADC of protein and energy in the reference diet were 97.2% and 70.1% respectively. In the experimental diets, the ADC of protein varied from 74.9% to 84.5% for fermented aquatic plants and from 51.1% to 60.4% for sundried aquatic plants. Protein digestibility was significantly higher in *Piaractus brachypomus* when diets containing the fermented plant material were offered. Among the plants, *Spirodela polyrhiza* and *Lemna minor* showed a higher digestibility than the water fern *Azolla filiculoides*. The ADC of energy did not reveal significant differences among treatments. According to these results the fermentation of aquatic plants is highly recommended for being used as a feed ingredient into fish diets.

The effect of the inclusion of fermented aquatic plants in low-fish meal content diets on the growth parameters of *Piaractus brachypomus* and *Oreochromis niloticus* was evaluated in **Chapter 3 and 4**, respectively. The growth trials were carried out during a period of 8 weeks. Practical diets were formulated with two inclusion levels (15% and 25%) of two selected groups of aquatic macrophytes, namely the duckweeds, *Lemna minor* and *Spirodela polyrhiza*) and the water fern, *Azolla filiculoides*. A total of four experimental diets and one reference diet were offered to fish with three replicates for each treatment. Trials with *Piaractus brachypomus* were conducted in 15 flow-through 250 litres circular plastic tanks in a closed recirculation system (Universidad de los Llanos, Colombia). Trials with *Oreochromis niloticus* were carried out in 15 glass aquaria with a volume of 250 litres of two similar recirculation systems (Humboldt University of Berlin, Germany). Fish were fed twice daily until apparent satiety.
The results showed no significant differences in the growth parameters among treatments for the species *Oreochromis niloticus*. The specific growth ratio (SGR) varied from 2.8 to 2.9 $\%.d^{-1}$. However, feed efficiency by *O. niloticus* decreased with the increasing inclusion level of aquatic plants in the diet, particularly in the WF25 diets. The experimental diets were not rejected by fish, but the increase in feed consumption and consequently the decrease of the

feed efficiency can be explained by the high fibre and ash content of aquatic plants which negatively affect the digestibility of diets.

Contrary, significant differences in the growth parameters were showed up for the species *Piaractus brachypomus*. Fish fed aquatic plants at 15% showed better growth parameters than fish fed the control and the 25% group. The SGR ($\%.d^{-1}$) varied from 3.6 in the 15% group to 3.3 in control diet and 3.2 in the 25% group. Fish fed the 25% group showed the lowest growth. Fermented DW and WF up to 15 % can be utilised in low-fish meal diets to reduce feeding costs without an impact on growth performance, feed conversion and animal welfare.

In **Chapter 5** the effect of the replacement of 15% of a commercial diet by fermented aquatic plants on the productive performance of Cachama (*Piaractus brachypomus*) and Nile tilapia (*Oreochromis niloticus*) in a traditional polyculture was evaluated. The growth trials were carried out during a period of 16 weeks. A common commercial fish feed (24% crude protein, 30% fish meal) was replaced at 15% by locally available aquatic plants which were previously fermented, the duckweeds *Lemna minor* and *Spirodela polyrhiza*, and the water fern *Azolla filiculoides*. Juveniles of Cachama blanca and Nile tilapia averaging 86.7 g and 39.6 g, respectively, were co-stocked in 12 experimental units (18 m^2 in area) at a total density of three fish m^{-2}. The species mixture consisted of 25% Cachama blanca and 75% Nile tilapia. No significant differences were observed between the control diet and the DW15 diet. Fish fed on WF15 showed the lowest WG of 382 g (*P. brachypomus*) and of 167 g (*O. niloticus*). The highest weight gain (WG) was obtained for fish fed DW15 resulting in 444 g and 176 g for *P. brachypomus* and *O. niloticus*, respectively.

The semi-extensive polyculture of *Piaractus brachypomus* and *Oreochromis niloticus* in earth-ponds based on the natural food offer and commercial feeds replaced by fermented aquatic plants at 15% inclusion level might signify an important reduction of the feeding cost in rural fish production.

According to the results obtained in this study, a feeding exclusively based on aquatic plants is not recommendable; but to combine them with other locally available by-products of agriculture or even with commercial diets might considerably reduce feeding cost and provide to the small-scale farmers the opportunity to compete in local markets.

Keywords: alternative fish feed, anti nutrients, aquatic plants, Cachama, duckweeds, digestibility, growth performance, lactic acid fermentation, Nile Tilapia, polyculture, water fern.

Zur Sicherung der Fischbestände muss die Aquakultur ihren Beitrag zur Weltfischversorgung weiter steigern. Solange jedoch die Fischfutter Produktion stark von der Gewinnung von Fischmehl abhängig ist, bestehen für die Aquakultur natürliche Begrenzungen und die Gefahr der Überfischung der Fischbestände bleibt erhalten.

Dies ist der Grund, weshalb die Ernährungswissenschaftler weltweit nach effizienten Substituten für Fischmehl suchen, welche dieses teilweise oder vollständig ersetzen können. Wenn das Wachstumspotenzial der Aquakultur ausgeschöpft werden soll, müssen beträchtliche Mengen von Nährstoffeinträgen in Form von Düngemitteln, Ergänzungsfutter oder vollständigen Aquakultur-Mischfuttermitteln auf einer nachhaltigen Basis verfügbar sein.

Aufgrund des gestiegenen Preises von kommerziellem Fischfutter, das traditionell auf Fischmehl basiert, sind Kleinproduzenten nicht in der Lage dieses zu erwerben. Daher ist es notwendig, ihnen alternatives Fischfutter zur Verfügung zu stellen, das auf lokalen Futtermitteln basiert. Diese müssen in ländlichen Regionen verfügbar und kostengünstig sein und zudem geeignet für das angemessene Wachstum der einheimischen Fischarten sein. Daher sind Proteine aus pflanzlichen Quellen eine logische Wahl für den Ersatz von Fischmehl in Diäten für herbivore- und omnivore Spezies, während Proteine aus tierischer Quelle als Alternativen für Fischmehl zur Fütterung von carnivoren Spezies geeignet sind.

Wasserpflanzen können eine bedeutende Nahrungsquelle für herbivore- und omnivore Fische sein. Aus früheren Untersuchungen ist bekannt, dass sie bis zu 25% der synthetischen Diäten und bis zu 50% der kommerziellen Futtermittel (35% Protein) ersetzen können, ohne nachteilige Effekte auf das Wachstum und die Körperzusammensetzung der Fische zu haben.

In vielen Teilen der Welt sind Wasserpflanzen weitverbreitet und können als Plage betrachtet werden. Dies ist in tropischen Zonen wie Kolumbien der Fall, wo diese aufgrund der hohen Sonneneinstrahlung und günstiger Temperaturen gut gedeihen.

Dennoch ist die Nutzung dieser Ressource als Fischfutter begrenzt. Die chemische Zusammensetzung der Wasserpflanzen ist in hohem Maße von der aquatischen Umgebung abhängig. Für Kolumbien gibt es nur wenige Laborstudien zur Nährstoffzusammensetzung der verfügbaren Wasserpflanzen. Außerdem ist die Nutzung dieser Pflanzen als Zusatz für Fischfutter durch eine Reihe antinutritiver Substanzen, welche das normale Fischwachstum negativ beeinträchtigen, begrenzt. Unterschiedliche Behandlungen der Pflanzen können den Anteil an antinutritiven Substanzen reduzieren. Es gibt wenige Informationen über das

Potenzial oder die Verarbeitung der lokal verfügbaren Wasserpflanzen, die in Kolumbien genutzt werden können.
Das Ziel dieser Dissertation war es, das nutritive Potential von Wasserpflanzen, die im ländlichen Kolumbien verfügbar sind, zu bestimmen. Die Wirkung der Behandlungen wie Sonnentrocknung oder Fermentierung zu bewerten und den Effekt ihrer Nutzung als Fischfutter auf das Wachstum von häufig kultivierten tropischen Fischen zu erfassen. Dazu wurden Rationen mit einem geringen Gehalt an Fischmehl (3%) und bis zu 25% der Wasserpflanzen an die Fischspezies *Piaractus brachypomus* und *Oreochromis niloticus* verfüttert.

In **Kapitel 1** wurden die Nährstoffgehalte der Wasserpflanzen *Lemna minor*, *Spirodela polyrhiza*, *Azolla filiculoides* and *Eichhornia crassipens* analysiert und ihre Siliereigenschaften untersucht.
Aquatische Makrophyten wurden als nicht kultiviertes Material von Gewässern im ländlichen Kolumbien gesammelt. Die analytischen Methoden für die Bestimmungen des Nährwertes und der Konzentration von antinutritiven Substanzen wurden gemäß Standardprozeduren durchgeführt. Die Fermentationscharakteristika wurden gemäß der DLG Methode untersucht, indem die Silagequalität auf der Basis einer chemischen Analyse bewertet wurde. Die Ergebnisse zeigten, dass die lokal verfügbaren Spezies einen akzeptablen Proteingehalt von 98 bis 243 g kg^{-1}, einen Rohfettgehalt von 13 bis 39 g kg^{-1} und einen relativ hohen Faser- (>12 g kg^{-1}) sowie Aschegehalt (>145 g kg^{-1}) aufweisen. Der Faser- und Aschegehalt der in dieser Untersuchung getesteten Pflanzen war vergleichbar mit Literaturwerten von verschiedenen Wasserpflanzen. Das Profil der Aminosäuren war in allen Pflanzen vergleichbar: mit 5,3 bis 6,3 g Lysin und 1,7 bis 2,0 g Methionin jeweils auf 100 g Protein. Die experimentellen Diäten, welche die Wasserpflanzen enthielten, erfüllten den Nährstoffbedarf der untersuchten Fischspezies.
Die Wasserpflanzen zeigten eine hohe Pufferkapazität von 70 bis 90 g kg^{-1} Milchsäure, einen geringen Gehalt an löslichem Zucker (<10 g kg-1) und einen hohen Feuchtigkeitsgehalt (>90%), der die Fermentierung hemmt. Durch Reduktion des Feuchtigkeitsgehaltes (<50%) und den Einsatz von Additiven (Laktobakterien und Molasse als löslicher Kohlenhydrat) konnte eine sehr gute Silagequalität erreicht werden (>90 Punkte der DLG). Der Fermentationsprozeß reduzierte den Fasergehalt in *Lemna minor, Spirodela polyrhiza* und *Azolla filiculoides* signifikant und auch die Konzentration von antinutritiven Substanzen war im fermentierten Material stark reduziert im Vergleich zu den frischen Pflanzen. Die

Konzentration an Trypsin-Inhibitor (<1,4 mg TI g^{-1}), Oxalaten (1,9 g kg^{-1}), Phytaten (2,0 g kg^{-1} Phytinsäure) und löslichen- und kondensierten Tanninen im fermentierten pflanzlichen Material überstieg nicht die Grenzwerte für Fische.

In **Kapitel 2** wurde die Protein- und Energieverdaulichkeit von sonnengetrockneten und fermentierten *Lemna minor, Spirodela polyrhiza* und *Azolla filiculoides* für die Fischspezies *Piaractus brachypomus* bestimmt. Die Nährstoffverdaulichkeit der Makrophyten wurde im Vergleich zu einer semisynthetischen Diät als Referenz bestimmt. 30% der Referenzdiät wurden jeweils durch sonnengetrocknete oder durch fermentierte aquatische Makrophyten ersetzt. Sechs experimentelle Diäten und eine Referenzdiät wurden während eines Zeitraums von 30 Tagen an je drei Gruppen von Fischen gefüttert. Im Anschluss wurden Kotproben über maximal eine Stunde gesammelt, bis die erforderliche Menge an Material für eine Analyse erreicht war. Das Experiment wurde in einem modifizierten Guelph-System durchgeführt. Die scheinbaren Verdaulichkeiten von Protein und Energie betrugen 97,2% und 70,1%. In den experimentellen Diäten schwankte die scheinbare Proteinverdaulichkeit zwischen 74,9% und 84,5% für die fermentierten und zwischen 51,1% und 60,4% für die sonnengetrockneten Wasserpflanzen. Diese Unterschiede waren signifikant. Unter den Pflanzen zeigten *Spirodela polyrhiza* und *Lemna minor* eine höhere Verdaulichkeit als der Wasserfarn *Azolla filiculoides*. Die Verdaulichkeit der Energie ergab keine signifikanten Differenzen zwischen den verschiedenen Behandlungen.
Diesen Resultaten zufolge ist die Fermentierung der Wasserpflanzen höchst empfehlenswert, wenn diese als Substitut für Fischdiäten vorgesehen sind.

Der Effekt der Substitution von fermentierten Wasserpflanzen in Diäten mit einem geringen Gehalt von Fischmehl auf die Wachstumsparameter von *Piaractus brachypomus* und *Oreochromis niloticus* wird in den **Kapiteln 3 und 4** beschrieben. Die Wachstumsversuche erstreckten sich über einen Zeitraum von 8 Wochen. Dazu wurden Diäten mit einem Anteil von 15 bzw. 25% fermentierter Makrophyten (Wasserlinsen *Lemna minor* und *Spirodela polyrhiza* DW bzw. Wasserfarn *Azolla filiculoides* WF) verfüttert.
Insgesamt vier Versuchsrationen und eine Referenzdiät wurden jeweils an drei Gruppen für jede Behandlung gefüttert. Die Versuche mit *Piaractus brachypomus* wurden in 15 Fließtanks zu 250 Liter in einem geschlossenen Kreislaufsystem durchgeführt (Universidad de los Llanos, Kolumbien). Die Versuche mit *Oreochromis niloticus* wurden in 15 Glasaquarien mit einem Volumen von je 250 Litern in zwei Kreislaufsystemen durchgeführt (Humboldt-

Universität zu Berlin). Die Fische wurden zweimal täglich bis zur augenscheinlichen Sättigung gefüttert.

Für die Spezies *Oreochromis niloticus* zeigten sich keine signifikanten Unterschiede der Wachstumsparameter. Die spezifische Wachstumsrate (SWR) variierte zwischen 2,8 und 2,9%.d^{-1}. Mit zunehmendem Anteil von Wasserpflanzen in der Diät sank die Futterverwertung bei *Oreochromis niloticus*, besonders in den WF25 Diäten.

Die Versuchsdiäten wurden nicht durch die Fische zurückgewiesen. Der hohe Faser- und Aschegehalt der Wasserpflanzen führte zur Senkung der Verdaulichkeit der organischen Substanz, diese wurde aber durch einen gesteigerten Futterverzehr kompensiert.

Im Gegensatz dazu zeigten sich signifikante Unterschiede in den Wachstumsparametern für *Piaractus brachypomus*. Eine jeweils 15%ige Substitution der Wasserpflanzen in den Diäten zeigte bessere Wachstumsparameter als die Kontrolldiät und die Diät mit 25% Anteil Wasserpflanzen. Die SWR (%.d^{-1}) variierte von 3,6 in der 15%-Gruppe über 3,3 in der Kontrolldiät und 3,2 in der 25%-Gruppe. Somit zeigten die Fische, welche mit 25% Wasserpflanzenanteil gefüttert wurden, das niedrigste Wachstum.

Als Ergebnis dieser Versuche ergab sich, dass fermentierte Wasserlinsen und Wasserfarne bis zu 15% in einer Diät mit einem niedrigen Gehalt an Fischmehl eingesetzt werden können, um die Futterkosten zu reduzieren, ohne einen negativen Einfluss auf das Wachstum, die Futterverwertung und das Wohlergehen der Tiere auszuüben.

In **Kapitel 5** wurden die Auswirkungen einer 15%igen Substitution einer kommerziellen Diät durch fermentierte Wasserpflanzen auf die Produktivität von Cachama blanca (*Piaractus brachypomus*) und Niltilapia (*Oreochromis niloticus*) in einer traditionellen Polykultur ausgewertet. Die Wachstumsversuche erstreckten sich über einen Zeitraum von 16 Wochen. Ein handelsübliches Fischfutter (24% Rohprotein und 30% Fischmehl) wurde zu 15% durch lokal verfügbare, zuvor fermentierte Wasserpflanzen ersetzt, die Wasserlinsen *Lemna minor* und *Spirodela polyrhiza* sowie den Wasserfarn *Azolla filiculoides*. Juvenile Cachama blanca und Niltilapia mit einem durchschnittlichen Gewicht von 86,7 g bzw. 39,6 g wurden gemeinsam in 12 experimentelle Einheiten (18 m²) bei einer Gesamtdichte von 3 Fischen pro m² gesetzt. Das Verhältnis der Fischspezies Cachama blanca und Niltilapia betrug 1:3.

Es wurden keine signifikanten Unterschiede zwischen der Kontrolldiät und der WL15-Diät beobachtet. Die mit der WF15-Diät gefütterten Fische zeigten den geringsten Gewichtszuwachs mit 382 g (*P. brachypomus*) und 167 g (*O. niloticus*). Den höchsten

Gewichtszuwachs erreichten die mit der WL15-Diät gefütterten Fische mit 444 g und 176 g für *P. brachypomus* bzw. *O. niloticus*.

Den Ergebnissen der vorliegenden Untersuchung zufolge, ist eine ausschließlich auf aquatischen Makrophyten basierende Fütterung nicht empfehlenswert. Indem sie jedoch mit anderen lokal verfügbaren Agrar-Nebenerzeugnissen oder sogar mit kommerziellen Futtermitteln kombiniert werden, könnten die Futterkosten erheblich reduziert werden und bäuerlichen Kleinbetrieben eine Möglichkeit zum Wettbewerb auf den lokalen Märkten eröffnen.

Schlagworte: alternative Fischfutter, antinutritiver Substanzen, Cachama, duckweeds, Milchsäure Fermentirung, Nil Tilapia, polyculture, Verdaulichkeit, Wachstum, Wasser fern, Wasserpflanzen.

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Background

Aquaculture is an activity that generates food, income and employment. Aquaculture may provide the means to revitalize rural living and to contribute substantially to an integrated rural development if it is applied responsibly and combined with agriculture and animal husbandry farm. In the international context, the inclusion of aquaculture in rural development goals has achieved considerable importance for its role as a protein source for food security of rural and marginal communities.

However, in the main less developed countries commercial aquafeeds are increasingly used for the production of both, lower value basic food fish species and, higher value commercial crop species for luxury markets. The technological development of the aquaculture industry is principally addressed to luxury markets and originated in industrialised nations. Thus, the results of this development have often been irrelevant to the needs of countries with no privileged social and economic conditions.

The rapid growth of aquaculture brought the challenge to ensure the sustainability of this industry, not only in terms of food production to improve food security on small, subsistence family farms but also in terms of preserving the aquatic environment. In the rural context it is necessary to include the aquaculture into integrated agricultural systems. This is only possible when aquaculture production is based on a highly efficient use of local resources.

For the development of a sustainable aquaculture and its integration to agricultural production systems is important to ensure the access to the sources of nutrients available in the rural environment. In fact, the key to sustainable production is directly related to assessing the potential of local resources in terms of quality, quantity and cost.

In tropical regions of South America aquatic plants are a resource widely available and a free source of highly nutritious feed. But no general criteria have been defined for their various uses as animal feed. This is because their nutritional characteristics are highly depending on the local available plant species, as well as, the environmental conditions and water quality where plants grow. Colombia has a great abundance of aquatic weeds that grow throughout the whole year. A few of them are used by local farmers as animal feed without any dietary fundament, and many remain underutilized and go to waste. Some of them, namely, *Lemna minor, Wolffia* sp *and Spirodela* sp*, Eichornia crassipes, Pistia striatotes* and *Salvinia* sp are widely distributed. Preliminary information on nutritional, chemical composition and antinutritional factors of this plant material is lacking. This information is necessary to incorporate this local resource into fish diets.

In Colombia aquaculture activity in the rural context is made by farmers, who annexed aquaculture to the farming activities that they normally practice. Aquaculture production is basically based on fish cultures in earthen ponds and floating cages for freshwater species as Tilapia (*Oreochromis* sp), Rainbow trout (*Oncorhynchus mykiss*) and Cachama blanca (*Piaractus brachypomus*). Particularly important is the culture of native species as Cachama blanca.

As commercial aquafeeds are very expensive and frequently not available to small-scale fish farmers, there is the need to develop or adapt simple, practical and cheap treatments for the use of locally available sources of nutrients as feed for rural aquaculture. It is more relevant in tropical countries as Colombia, where the diversity of nutrient sources is enormous. Because of the high productivity of this plant material and their potential as alternative feed, the present research is focused on the use of aquatic plants as a practical and environmental friendly way to develop feeding strategies for common cultured fish in the rural Colombia.
This work not only sets out the nutritional characteristics of aquatic plants and their suitability as fish feed, but also provides information on their handling and processing in farm.

Rationale of the study

Aquatic plants are considered to be an important source of nutrients for fish and in many cases could replace up to 50% of commercial feeds without adverse effects on fish growth and body composition. Chemical content of aquatic plants is highly affected by the aquatic environment in which they grow; therefore their use is recommendable in each case on a local level. In Colombia there are only few studies on the evaluation of the nutritional characteristics of locally available aquatic plants. Thus, the evaluation of the nutritional quality and processing of this plant material through simple and cheap methods as fermentation and sun drying can be very useful for converting them into a valuable feed ingredient for a sustainable feeding strategy.

General objective

To evaluate the effect of aquatic plants treated by sun drying and by fermentation on the performance of common cultured tropical fish (*Oreochromis niloticus, Piaractus brachypomus*) fed low fishmeal diets.

Specific Objectives

1. To evaluate the nutrient composition, potential and characteristic of lactic acid fermentation of selected aquatic plants to identify the best suitable sources.
2. To analyse the changes in composition of selected aquatic plants after fermentation with main focus on antinutrients.
3. To determinate apparent digestibility coefficients (ADCs) of dry matter, protein and energy of sundried and fermented aquatic macrophytes for the Amazonian fish Cachama blanca (*Piaractus brachypomus*).
4. To evaluate the growth response of the Amazonian fish Cachama blanca (*Piaractus brachypomus*) and the tropical fish Nile Tilapia (*Oreochromis niloticus*) fed on fermented aquatic macrophytes as alternative feed source.
5. To evaluate the growth and productive performance of Nile Tilapia (*Oreochromis niloticus*) and Cachama blanca (*Piaractus brachypomus*) in a traditional polyculture fed on commercial aquafeed partially replaced by fermented aquatic macrophytes.

Experimental approach

The experiments in this study were conducted at the *Institute for Animal Breeding in the Tropic and Subtropics of the Humboldt University of Berlin* (Germany), at the *Instituto de Acuicultura de la Universidad de los Llanos* (IALL) in Villavicencio (Colombia), and at the *Instituto de Investigaciones Tropicales de la Universidad Del Magdalena* (INTROPIC) in Santa Marta (Colombia).

Preliminary research on plant material was carried out according to guidelines for the use of no conventional feed material. Aquatic macrophytes were harvested as wild or uncultivated material from water bodies at three locations in rural areas from Colombia during the months June to August in 2008. Analyses of the nutrient content and antinutrients concentration were realized following standard procedures. The fermentation characteristics of the aquatic macrophytes were determined according to the DLG-method (Deutsche Landwirtschafts-Gesellschaft – German Agricultural Society) which is commly used to assess the fermenting quality of forage ensilage on basis of a chemical evaluation.

Determination of apparent digestibility coefficients of gross nutrients and energy of aquatic macrophytes for herbivorous-omnivorous tropical fishes were carried out using a semi-purified diet as reference. In all the cases 30% of the reference diet was replaced by untreated and treated by lactic acid fermentation aquatic macrophytes (Lemna, Spirodela and Azolla). Six experimental diets and one reference diet were offered to triplicate groups of fishes during a 4 wks period. After that, faecal samples were collected with a maximal interval of one hour until the required amount for analyses was obtained. Experiment was conducted in a modified Guelph system.

The growth trials were carried out during a period of 8 weeks to test the growth performance of Nile Tilapia and Cachama blanca. Practical diets were formulated at two inclusion levels (15% and 25%) of two selected groups of aquatic macrophytes, the namely duckweeds (*Lemna minor* and *Spirodela polyrhiza*) and the water fern *Azolla filiculoides*. A total of four experimental diets and one reference diet were offered to triplicate groups of fishes. Experiments were conducted in 15 flow-through 250 litres circular plastic tanks in a closed recirculation system for *Piaractus brachypomus* and in 15 glass aquaria with a volume of 250 litres for *Oreochromis niloticus*. All the fishes were fed twice daily at 9 am and at 3 pm until apparent satiety. Faecal samples were collected after finished the growing experimental period. Water quality parameters from each tank or aquaria were monitored once weekly throughout the experimental period.
Apparent digestibility coefficients of gross nutrients (ADCs, %), specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR, %), protein efficiency ratio (PER, %) and apparent net protein utilization (ANPU, %) were calculated using standard methods. Samples of intestinal tract and the stomach were taken for histological analysis.

In order to know the effect of using aquatic plants as feed under real growing conditions of fish, one last experiment was conducted in a traditional polyculture. A common commercial fish feed (24% crude protein, 30% fish meal) was replaced at 15% by locally available aquatic plants, the duckweeds *Lemna minor* and *Spirodela polyrhiza,* and the water fern *Azolla* *filiculoides*. Plants were previously fermented as indicated in the prior experiments. The growth performance and productive parameters of Tilapia and Cachama blanca in a traditional polyculture was evaluated.

Experimental diets consisted of a reference semipurified diet for determination of digestibility coefficients, a basal practical diet for evaluation of growth performance and finally a formulated diet consisted on a commercial fish feed containing 24% crude protein. Tested diets were elaborated by replacement or supplementation of the test ingredients in the reference, basal and commercial diets at previously determined inclusion levels.

Thesis outline

General Introduction gives an overview of the main topic of the study, describes the study problem and its rationality as well as the general and specifics objectives of the thesis.

Literature review gives information about rural aquaculture and its role in the rural regions of South America. Particularly it deals with the importance of fish feeding and the need for cost-effective and balanced fish feeds.

Chapter 1. This chapter consists in the evaluation of the nutritional characteristics of some of the most common aquatic plants available in the tropical regions of Colombia (*Lemna minor, Spirodela polyrhiza, Azolla filiculoides,* and *Eichhornia crassipens)*. The importance and effects o the fermentation process on the nutritional value, the silage properties (fermentability coefficients) and the final products of the lactic acid fermentation of locally available aquatic plants were examined as well.

Chapter 2. Based on the results obtained about the nutritional characteristic of aquatic plants and the information obtained from the literature, this chapter discusses the digestibility of the sundried and fermented *Lemna minor, Spirodela polyrhiza* and *Azolla filiculoides* for the Amazonian fish Cachama blanca. Fish were fed a semipurified reference diet. Each aquatic plant was replaced at 30% inclusion level and as an indicator chromic oxide was added at 5%. Results were used to formulate the practical diets for the growth trials in chapters 5 and 6.

Chapter 3. This chapter deals with the 8 wks growth trials for the species *Piaractus brachypomus*. It discusses the effects of the fermented aquatic macrophytes inclusion (15 and 25%) in low-fish meal diets (3% fishmeal) on the growth performance, feed utilisation and digestibility. A total of five experimental diets were formulated (35% crude protein) according to the nutritive characteristics of the plants and the previously determined digestibility coefficients.

Chapter 4. This chapter deals with the 8 wks growth trials for the species *Oreochromis niloticus*. The effect of a diet composed principally of plant ingredients and with a very low fish meal content (<3%) was evaluated versus four diets containing fermented aquatic macrophytes at two inclusion levels (15 and 25%). Aquatic plants were offered as fermented duckweeds (*Lemna minor* and *Spirodela polyrhiza*) and fermented water fern *Azolla filiculoides*. Variations in growth performance, feed efficiency, carcass composition and physiological parameters are discussed in detail.

Chapter 5. This chapter deals with the results of a 120 days on-farm evaluation of the polyculture of Cachama blanca and Nile tilapia raised in earth ponds. Three experimental diets were offered in this trial: a commercial diet (CD) with 24% crude protein; commercial diet replaced by 15% fermented duckweeds (DW15), and commercial diet replaced by 15% fermented water fern (WF15). Growth performance and productive parameters of both species in a polyculture are discussed in detail.

General discussion deals with the most important findings on the potential of treated aquatic plants as fish feed and their effect on the performance of common cultured tropical fish fed low fishmeal diets and replaced commercial diets. It briefly summarises the relationship among the results of the different chapters and presents future research directions and general conclusions of the work.

Research on fish nutrition in the 50's and 60's was focused on the anatomy of the digestive tract and very limited to aspects related to the digestive physiology and feeding of animals in the wild (Guillaume et al., 2004). With the rapid growth of aquaculture in the world there was a need to replace or supplement the natural food with specific diets that raised the growth rate of different fish species in captivity. To achieve this objective it was essential to know the nutritional requirements of the fish. Thus, feeding and nutrition became one of the most important fields for aquaculture production.

Over the years, aquaculture got higher importance in the global food production industry because it has proven to be a valuable source of high quality protein for the population's food security as well as a profitable activity. Unfortunately, aquaculture is highly depended of formulated feed. The main component for fish feed formulation is fishmeal which is obtained from fish processing. By reducing fishing and increase demand for cultured products, the price of fishmeal has been increased continuously and subsequently the price of fish feed also increased. So, aquaculture feeds is currently one of the most expensive animal feeds (Ogunkoya et al., 2006).

Aquaculture has still the potential to provide high quality protein for securing food demand in rural areas and to generate income also for small-scale farmers. To maintain the profitability of aquaculture feed costs must be diminished. On the one hand, low feed costs could be achieved by increasing the efficiency of proteins in diets, which are the most expensive element of feed. On the other hand, it must be combined with feeding strategies for the different fish species and with an efficient use of the available resources. Thus the need to seek alternative sources of protein to substitute fishmeal protein in aquaculture feed has become a global challenge.

Aquaculture of Tropical Fish Species (The Case of Colombia)

Colombia has not been indifferent to the global trend of aquaculture and its participation in this activity has grown in the last years. According to FAO (2012) the total aquaculture production in Colombia reached 80.367 tonns in 2010. In Colombia aquaculture is focused on the cultivation of fish and crustaceans, mostly those species are freshwater fish such as Tilapia

(*Oreochromis* sp), Rainbow trout (*Oncorhynchus mykiss*), and Cachama blanca (*Piaractus brachypomus*), which represent about 96% of the total national production (Cruz-Casallas et al., 2011). These three species have high commercial potential, and are an alternative to basic food production in the country, but of them Cachama blanca is the only native species.

Cachama blanca is an Amazonian fish of the family Characidae and belongs to the genus Piaractus. It is widely known in South America, being abundant in the basins of the Amazon and Orinoco rivers (Machado-Allison, 1982; Martins de Proenca and Leal, 1994). It lives in water temperatures of 26 ± 1.2 ° C, pH 7.3 ± 0.2, hardness> 40 ppm and nitrites and ammonium concentration <0.02 ppm. (Arias and Vasquez-Torres, 1988). Juveniles usually have bluish gray coloration with reflections on the back and flanks, the abdomen is white with light orange spots (Figure 1). It may reach a weight of 20 kg and a length of 85 cm. Adult males of *P. brachypomus* are suitable for reproduction after 3 years whereas adult females after 4 years when weighing 3 to 4 kg (González, 2001).

Figure 1: Cachama blanca (*Piaractus brachypomus*). Source: Aquaculture Aguaverde

Cachama's natural eating habit is omnivorous, frugivorous-prone herbivores. Its diet is based mainly on fruits, seeds, parts of aquatic macrophytes, large zooplankton organisms, molluscs, crustaceans and insect larvae (Arias, 1988; Diaz and Lopez, 1993). They have a terminal mouth and mandibular teeth, which allows grinding different hardly feed. Cachama's intestine is physiologically adapted to this feeding behavior (González, 2001). Although Cachama is a non filter feeder fish, its omnivorous habit qualifies it to accept different types of natural foods, achieving high rates of feed conversion (Espejo, 1984).

Cachama blanca production in Colombia is very recent and its cultivation has been developing in the last two decades (Vasquez, 2004). Thus, Cachama blanca is a relatively new species for aquaculture; its production has grown from 3181 tons in 1995 to 12022 tons in 2003 (Salazar, 2006). The commercial importance that this species has reached is due to the excellent quality and flavor of its meat, which gives good market acceptance (Bello & Gil, 1992).

Beside Cachama blanca (*Piaractus brachypomus*) the only native fish species commercially farmed in Colombia are Yamú (*Bricon amazonicus*) and Bocachico (*Prochilodus magdalenae*) (Salazar, 1999, CCI, 2006). The low availability of the knowledge on native fish species in Colombia reduces its applicability to the productive systems (Salazar, 2005).

Tilapia, meanwhile, is the second most important group of fish for worldwide aquaculture and occurs in all five continents. Among all species, Nile tilapia (*Oreochromis niloticus*) (Figure 2) stands out as the most important in production volumes (El-Sayed, 2008). In view of the wide distribution that tilapia already has in Latin America and its acceptance by consumers, there is a growing trend to develop modifications and improvements in its cultivation techniques. In the world is produced about 2 million tonnes Tilapia, with China being the largest producer. Production in Colombia comes to 24.500 tons in 2005. There are more than 96 species of Tilapia, and that there are several commercially cultivated around the world. About 80% of world production is contributed by the Nile tilapia (Ocampo, 2007).

Figure 2: Nile tilapia (*Oreochromis niloticus*) Source: Aquaculture Aguaverde

Tilapia species are tolerant of high temperatures, low oxygen and high levels of ammonia. Tilapias are resisting to high salinity, up to 20 ppm and have little tolerance to low temperatures, which becomes a serious problem for their culture in temperate regions. Reproduction is inhibited when temperatures are below 20 ° C and for growth; it is desirable 29 and 31 º C. When the temperature exceeds 37-38 °C problems can also occur due to stress. This species survives at concentrations of 0.5 $mg.l^{-1}$ dissolved oxygen levels considered low compared to other species. It grows best in water of pH neutral or slightly alkaline (Lim and Webster, 2006).

The Nile tilapia is a filter feeding fish and feeds into the natural environment of a variety of food as plankton, benthos, invertebrates, fish larvae, detritus, decaying organic matter, etc. In ponds with external supplement feed, natural food can represent between 30 and 50% of total food consumed. In polyculture with other fish, natural food is considered very important for its growth (Karplus et al., 1996).

In 2008 Tilapia reached 58% of national fish production in Colombia, followed by Cachama blanca with 23%, Rainbow trout with 8% and others species with 11% of the total production (ENA, 2008).

Aquatic plants and its potential as fish feed ingredient

Fishmeal has been largely used as the main protein ingredient of the diets for aquatic and terrestrial organisms, but due to its high cost and the increasing demand of feed in the aquaculture sector efforts have been made to find alternative sources of conventional and unconventional proteins. Currently plant proteins are expected to be an appropriate feed ingredient in the aquaculture; hence a large number of institutions are evaluating the potential of alternatives plant proteins in fish diets.

Aquatic macrophytes are highly productive plants. They are characterized by their wide distribution and habitat. Due to their accelerated growth, several studies of these plants are addressed to their control or alternative uses because of their high nutritional quality (Kalita, P. et al., 2007, Setlikova and Adamek 2004, Yılmaz et al., 2004, El-Sayed, 2003, Pipalova 2003; Bairagi et al., 2002). Aquatic plants can be used as fodder for livestock and herbivorous fish species in fresh or processed form. They can also be processed to be included as an ingredient in animal feed. Due to their chemical composition, aquatic plants can even be partial substitutes for protein concentrates that are part of the rations of fish and other farm animals. However, the feasibility of their use depends on the cost incurred in the collection and processing.

There is a wide variety of aquatic plants in the tropical world. Most of them may be usable as a nutrient source, including *Lemna minor, Spirodela polyrhiza, Azolla* sp., *Pistia stratiotes* and *Eichornia crassipes,* among others. Here are described those which are available in Colombia throughout the whole year and are found most frequently in aquatic ecosystems where aquaculture is practiced.

One of them is the genus Azolla, it is a tiny aquatic fern, which float freely on the water surface, and which is spread throughout all tropical regions (Espinas et al., 1979). Azolla consists of a short stem possesses branched roots hanging down in the water. Each sheet is bilobed, the upper lobe contains green chlorophyll while the lower lobe is colorless. Under certain conditions, there is also a pigment anthocyanin, which gives to the fern a color between red and brown. This coloration is associated with over-fertilization of the reservoir water, pollution or excessive sunlight (Ly, 2002).

Azolla has the ability to fix atmospheric nitrogen through its symbiosis with a cyanobacterium that fixes it, the *Anabaena azollae* (Peters et al., 1982, Calvert et al., 1985). The *Anabaena azollae*, who lives in the cavities of the fern fronds, is able to use its own photosynthetic energy to fix atmospheric nitrogen and produce ammonia, which is used by the Azolla to meet their own requirements of nitrogen. Still, some environmental factors such as soil and water conditions as well as cultivation techniques are an important influence on the nutrient content of Azolla (Naegel 1998).

Azolla is often used as green manure in rice cultivation, and its potential as animal feed has been relatively poor investigated (Alcántara and Querubín 1982, 1985). In the Philippines it was studied its possible use as feed for monogastric animals, whereas in some Latin American countries such as Mexico, Cuba and Colombia, it was investigated its possible use as swine feed. A review of the studies on this aquatic plant as ingredient into fish feed show that *Oreochromis niloticus* fed with *Azolla pinnata* grows well at levels up to 42% of inclusion level in diets with 35% crude protein, whereas other studies reported a marked reduction of growth of Nile tilapia fed Azolla. However, most of the studies report that the inclusion of aquatic macrophytes at level up to 25% supported fish growth, when fish meal content ranged between 7.5 to 22% in the diet (Fasakin et al, 1999; Kalita et al, 2007 and Abdel-Tawwab, 2008).

Other aquatic plants which are well known and used in fish feed are the Lemnaceae or duckweeds. They are small floating macrophytes that grow in stagnant or slow-flowing waters and are worldwide distributed. They grow very fast, and are in their natural habitat, a food appreciated by fish, wading birds, and aquatic rodents (Boyd 1968).

The Lemnaceae has four genera: Spirodela, Lemna, Wolffiela and Wolffia, and about 40 species (Hillman 1961). The *Wolffia arrhiza* is the lowest of all Lemnaceae, which has the size of a pinhead; it has been used as a highly nutritious vegetable by the Burmese, Laotian and Thai North communities for many generations (Krachang and McGarry 1971). Lemna easily reproduced by vegetative propagation form large masses or colonies which are distributed as a sheet or film on the water surface (Hillman, 1961). The Lemnaceae can double their biomass in a period of two or three days under favorable environmental conditions, and so it has proved to be gained yields 10-13 t DM / ha per year in small lakes systems, while in outdoor tanks yields are close to 20 t DM / ha per year (Said et al. 1979).

Many authors consider duckweeds as a high nutritional plant when used as fresh feed. Studies conducted by Hassan and Edwards (1992) found that Lemna is an appropriate supplemental feed for herbivorous fish such as Tilapia *Oreochromis niloticus*. In their study duckweed is used as flour and fresh feed, latter presented the highest nutritional value. Several studies have showed that fresh Lemna can replace up to 50% of protein diet of conventional ingredients as fishmeal and soybean meals, which are commonly used into fish diets. The same was observed for *Oreochromis mossambicus* and *Oreochromis hornorum* fed Azolla (Ponce et al., 2004).

Aquatic plants have not been assessed as dietary ingredients in diets for Cachama blanca (*Piaractus brachypomus*) yet.

Factors limiting nutritional value of feed (Antinutrients)

Tropical areas are rich in nutritional potential resources (e.g. aquatic plants), which can be an alternative for aquaculture feed. However, this apparent high availability of plant as ingredients for animal feed cannot be used to its full potential by limiting effect imposed by the so-called anti-nutritional factors (ANF). Particularly in the case of monogastric animals, lacking the protective action that provides the bacterial degradation, the problem of their use is higher but not exclusive.

The effect of the ANF is not only to interfere with the absorption of nutrients but in several important cases it promotes the loss of endogenous protein and in some cases causes damage to the organs (hepatopancreas and kidney) of the animal that consumes them. In conventional feeding animals and from a purely biological perspective, it has been noted that its use produces the waste of potentially useful protein which undermines the sustainability of production. It is due to the substantial losses of N and consequently, to the negative environmental impact caused by animal waste.

Antinutritional factors are divided into four groups: 1) Factors affecting protein utilization and digestion as protease inhibitors, tannins and lectins. 2) Factors affecting the utilization of minerals as oxalates, phytates, gossypol, among others, 3) antivitaminic Factors, 4) impure substances as mycotoxins, mimosinas, nitrates alkaloids and saponins (Francis et al. 2001).

The antinutritional factors affecting the aquaculture production are they which have an adverse effect on the growth of cultured organisms. The most commons are the protease inhibitors, which are proteinaceous compounds inhibiting the action of digestive enzymes whose action is focused on dietary proteins. The best known are those that react with serine proteases such as trypsin and chymotrypsin. A total of ten families of protease inhibitors have been identified according to the sequence of amino acids that comprise, three of them are the most widely distributed, namely, families Bowman-Birk, Kunitz and Potato-1. They are found in most legumes and due to their high content of cystine, they provide up to 40% of the sulfur amino acid content of the total protein (Kakade et al., 1969, quoted by Liener 1989 and Gueguen et al. 1993).

Protease inhibitors have a growth depressant effect in animals due to a negative feedback mechanism and the simultaneous inactivation of trypsin, thereby causing the release of cholecystokinin (CCK), a hormone of the intestinal mucosa which stimulates pancreatic acinar cells to release more other enzymes such as trypsin and chymotrypsin, elastase, and amylase. So in addition to the underutilization of dietary protein, it leads to the loss of endogenous protein rich in sulfur amino acids and the consequent depression of growth (Liener 1989).

Other important antinutritional factor is tannin. Tannins are defined as natural polyphenolic compounds, soluble, complexed with proteins, carbohydrates and other food polymers. They can precipitate alkaloids, gelatin and other proteins in aqueous solutions (Huisman and Tolman 1992, Jansman 1993). Because of their structure and reactivity to hydrolytic agents tannins are classified into two groups: hydrolyzable tannins and condensed tannins. The first are easily hydrolyzable by acid or enzyme. Whereas, condensed tannins are polymers flavonoids, which are not susceptible to hydrolysis but can be degraded oxidatively in strong acids to produce anthocyanins.

Tannins affect negatively the utilization of nutrients in monogastric animals. Tannins can inhibit digestive enzymes and form complexes with mucous membranes, resulting in increased endogenous losses and damage thereto. They decrease digestibility of nitrogen nutrients and energy. Furthermore, it has been reported that the hydrolyzable tannins could cause toxic effects to systemic level. Particularly important are also the negative effects on the liver (Huisman and Tolman 1992; Jansman 1993, Butler and Bos 1993).

In general, antinutritional factors (ANFs) have been identified in some traditional plant-derived feed ingredients, as soybean meal which contains substances as trypsin inhibitors, lectins, oligosaccharides, soy antigens, phytoestrogens, phytic acid, antivitamins, and saponins (Francis et al. 2001; Dersjant-Li 2002). In aquatic plants this information is lacking.
Over the last years studies conducted on the use of aquatic plants in fish feeds have reportd varying and conflicting results. It may be explained bythe different content of antinutrients causes by environment, plant species and treatments, their effects and methods for their reduction in aquafeeds limit the interpretation of the data.

Process to improve the nutrient availability of feed (Fermentation Methods)

Fermentation processes are used as an alternative to preserve feed quality and also to improve the nutrient availability of feed ingredients by the biosynthesis of vitamins, essential amino acids and proteins. By the degradation of the complexes structure between ANF and nutrients, more digestible protein and fiber, and micronutrients delivered.
To induce a good fermentation is necessary to increase the sugar content, either by adding them directly (e.g. using molasses) or introducing enzymes that can release other sugars in the feed.
The fermentation process has two major phases. The first phase is aerobic and occurs in the presence of oxygen waste. This oxygen is consumed by the living material during respiration. The second phase is anaerobic and begins when the available oxygen is used by the anaerobic bacteria which multiply rapidly at the beginning of the fermentation process.

Fermentation can be affected by several factors as the content of soluble carbohydrates. Microorganisms use soluble carbohydrates to obtain energy for growth. The main sugars are fructose, glucose, and sucrose. A minimum of 6 to 12% of the soluble carbohydrate is required for the fermentation. Also moisture content may affect the fermentation, with low concentrations of moisture, lactic acid bacteria become more tolerant. The type of bacteria is also important for fermentation, it occurs when lactic acid is produced by the predominant bacteria (*Lactobacillus acidophilus, L. casei, L. plantarum, L. fermentum, L. brevis*).

The lactic acid fermentation is carried out by lactic acid bacteria whose activity is developed in the absence of oxygen (anaerobic), and is expressed in the transformation of the sugars present in the plant into lactic acid, ethanol and carbon dioxide. Lactic acid is a colorless compound of formula CH3CHOHCOOH. It occurs under optically active dextrorotatory and levorotatory, often referred as acid D - lactic acid and L - lactic. In its natural state is an optically inactive mixture composed of equal parts of both D and L forms, known as a racemic mixture.

Aquatic plants as other plant materials generally possess antinutritional substances that restrict their use as feed. Fermentation can help to improve their nutritional value for fish (El-Sayed 2003, Bairagi et al., 2002). Aquatic macrophytes have been poorly investigated as fermented product and there is very few information on their fermentation characteristics.

Sustainable rural aquaculture

Aquaculture plays an important role in current and future world food production. Especially, aquaculture should continue to be an alternative to the food security of people in rural areas. To ensure the continuity of this activity it is necessary to redirect research towards sustainability of rural aquaculture.

Rural aquaculture and industrial aquaculture respond to different logics and therefore the strategies for their development are also different. The term "rural" can cause some confusion, since almost all fish culture is practiced in rural areas of the world. The term is actually related to the scale of intensification of the farming system. Rural fish farming for example can be defined as a productive activity practiced by farming families through an extensive or semi-intensive culture system for domestic consumption or partial marketing (Edwards and

Demaine, 1997). So, in the rural aquaculture the culture of native fish species and the practices of extensive or semi-intensive polyculture as well as the performance of complementary feeding strategies can provide in many cases a sustainable solution to the aquaculture production.

The main reason for using native species in rural aquaculture is that they are best suited to the characteristics of the local aquatic environment. Otherwise, introduced species represent a threat to the native wildlife and the ecological balance of the ecosystems.
Likewise, the use of extensive to semi-intensive culture systems usually involves less sophisticated methods, depends on natural food and is less expensive than intensive systems. Besides, polyculture maximizes the production through the cultivation of an appropriate combination of fish species with different feeding habits, allowing better use of the natural food available in the pond (De la Lanza-Espino et al., 1991). Polyculture began over a thousand years ago in China, where they have spread throughout Southeast Asia to other regions of the world. In tropical areas, a polyculture efficiently managed can produce up to 8000 kg/fish/h/year (Bardach et al., 1972, Landau, 1992).

The adoption of these practices combined with water fertilization, mixed-feeding schedules, reduced feeding rates and natural food-base ponds are very recommendable for the reduction of feed costs in aquaculture (El-Sayed, 2008) as well as the negative impact of this activity may have on the environment.

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CHAPTER 1.

Fermentation properties and nutritional quality of selected aquatic macrophytes as alternative fish feed in rural areas of the Neotropics.

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Abstract

In order to facilitate the economical culture of fish in rural areas of the Neotropics, the potential of locally available aquatic macrophytes (*Lemna minor, Spirodela polyrhiza, Azolla filiculoides* and *Eichornia crassipes*) from northern Colombia as alternative fish feed was studied. Considering the importance of fermentation in the improvement of nutritional value of non-conventional feeds, the fermentation properties (Trial I) and the effects of anaerobic fermentation on the nutritional quality of the selected aquatic plants were evaluated (Trial II). Results of the first trial indicated that although the fermentability coefficients (FC) of the selected aquatic macrophytes revealed a heavily fermentable material (FC< 35), the use of bacteria inoculants (*Lactobacillus plantarum*) and molasses (150 g/kg) resulted in a good silage quality. Results of the second trial showed that lactic acid fermentation positively affected the nutritional quality of the plants; since the concentration of some antinutritional substances and crude fibre content (P > 0.05) were reduced. The tested aquatic macrophytes may be used as alternative fish feed, but it is highly recommendable to ferment them if included in fish diets.

Key words: alternative fish feed, antinutritients, aquatic plants, lactic acid fermentation.

Introduction

Aquatic macrophytes are one of the most common plant materials in the Neotropical floodplain systems. In the Amazon floodplain macrophytes flooded forest contributes 60 % of the net primary production (Leite et al., 1999), whereas in the Orinoco, they are also reported to be highly productive and to increase rapidly during high water (Vásquez 1989). In other freshwater ecosystems within the tropics, such as shallow lakes or small perennial water bodies, they grow rapidly and remain during the whole year. If they are not harvested or properly managed, they can lead to an eutrophic effect in the water (Xie et al., 2004).

Due to their abundance, many researchers are looking for methods to control the weeds effectively and others to convert them into utilizable resource (NAS 1976; Brabben 1993; Rodriguez and Preston 1996; Franklin et al., 2008) putting them in industrial processes and also into rural uses (Leng, 1999; Xiao et al., 2009; Dordio et al., 2010). In fact, in sustainable production systems, they have been frequently reported as suitable alternative animal feed

(Pipalova 2003; Setlikova and Adamek 2004; El-Sayed 2003; Bairagi et al., 2002; Yılmaz et al., 2004 and Kalita et al., 2007). However, research conducted to evaluate the potential of aquatic plants as feed should be carried out on a local basis, since their nutrient characteristics are highly variable depending on their particular growth conditions (Boyd 1971).

As other plant materials, aquatic macrophytes possess antinutritional substances that restrict their use as feed. To improve their nutritional value for fish and subsequently to increase their incorporation level into fish diets they are commonly fermented (El-Sayed 2003, Bairagi et al., 2002). Although fermentation is one of the oldest processing techniques practiced in the tropics for preservation of the nutritional quality of feed material, aquatic macrophytes have been poorly investigated as fermented product and there is scarce information on their fermentation properties.

Since this information is essential to assure silage of good quality and subsequently to increase the potential of aquatic macrophytes as fish feed in the rural areas, two separated trials were conducted to evaluate the fermentation properties (Trial I) and the effect of anaerobic fermentation on the nutritional quality and the content of antinutritional substances (Trial II) of *Lemna minor, Spirodela polyrhiza, Azolla filiculoides* and *Eichornia crassipes*.

Materials and methods

Fermentation properties of the selected aquatic plants (Trial I)

The aquatic macrophytes *Spirodela polyrhiza*, *Azolla sp.* and *Eichornia crassipes* used in Trial I were obtained from a commercial distributor (Wakus GmbH Berlin, Germany). *Lemna minor* was obtained from the Botanical Garden of Berlin. The plant material was washed, oven dried and stored in plastic bags for preliminary analysis in laboratory. *Eichornia crassipes* was separated in leaves and roots and chopped into small parts before to be dried. For fermentation, freshly harvested plants were mixed with a part of the oven dried aquatic macrophytes of the same sample until about 350 to 450 g/kg dry matter content was obtained. A total of 8 mixtures, two replicates per plant species, were used for lactic acid fermentation by addition of the commercial silage inoculants and of molasses at 15 % as water soluble carbohydrate (WSC) source. Afterwards, the mixtures were vacuum packed into gas tight plastic bags according to the method described by Johnson et al. (2005) and maintained in an incubator for 60 days at 25°C.

Bacterial culture

For lactic acid fermentation, the commercial silage inoculants BIO-SIL® based on the LAB strain *Lactobacillus plantarum* DSM 8862 and DSM 8866 (Dr. Pieper Technologie-und Produktentwicklung GmbH, Germany) was used and prepared according to the manufacturer's information. The inoculants solution was used on a fixed dose of 2 ml/kg of plant material for a final inoculation rate of 3 x 10^5 cfu g^{-1}.

Factors affecting fermentation and end-products of the lactic acid fermentation

The raw plant material were preliminary analyzed in triplicates for determination of the buffering capacity (BC) by electrometric titration (Automatic Titrator Typ. AT 3 equipped with a glass electrode), nitrate content by potentiometric (ion selective electrode) and water-soluble carbohydrates (WSC) content by anthrone method (Lengerken and Zimmermann 1991). The ratio WSC/BC was used to estimate the fermentability coefficients (FC).

After fermentation the duplicate silages were analyzed in triplicates for pH value, acid fermentation concentrations by HPLC following the method of Weiss and Kaiser (1995), alcohols by chromatographic (GC) methods and ammonia by Conway method (Lengerken and Zimmermann 1991). The evaluation of the fermentation quality of the aquatic macrophytes silages was done on the basis of the chemical investigation according to the DLG (Deutsche Landwirtschafts Gesellschaft e.V./ Internationally acknowledged German Agriculture Society) guidelines for silage quality proposed by Weißbach and Honig (1992).

Calculations

The fermentability coefficient (FC) was used to judge the ensiling potential and calculated according to the parameters on the silage fermentation: FC=DM (%) + 8 WSC/BC (Schmidt et al., 1971). Ensiling potential according to FC values are described for FC>45 as easily fermentable, for FC=35-45 as medium fermentable and for FC<35 as heavily fermentable. The minimum dry matter content (DM_{min}) was calculated following the equation proposed by Kaiser et al. (2002) as a function of nitrate content. As the DM_{min} content considered acceptable for ensiling in farm scale is 200 g kg^{-1}, whereas a DM content of 450 g kg^{-1} is considered as inhibitory limit value for clostridia g kg^{-1} growth in anaerobic conditions (McDonald et al., 1991), samples were set to be ranged from 350 to 450 g kg^{-1} DM content.

Effect of fermentation on the nutritional quality of the selected aquatic plants (Trial II)

The aquatic macrophytes *Lemna minor*, *Spirodela polyrhiza*, *Azolla filiculoides* and *Eichornia crassipes* used in Trial II were harvested as wild or uncultivated material from water bodies at northern Colombia. After collecting and taxonomical identification, raw plant material was treated according to the procedures described above. A total of 12 mixtures, three replications per plant species, were prepared for fermentation. A sample of each mixture was taken before the vacuum packing to be analyzed for proximate composition. After 60 days of fermentation samples were opened and again analyzed. Proximate analysis of the unfermented and fermented aquatic plants was performed following the AOAC (2005) procedures.

Chemical analysis

Samples of unfermented plant material were sent to a private laboratory for testing mineral and heavy metal content. Determination were done by atomic absorption spectrophotometry according to the AOAC methods 985.35 and 983.27 (AOAC 1995) and to the AOAC methods 995.11 and 999.11 (AOAC 2005). For amino acid composition samples of the unfermented material were also sent to the Nutrition and Food Research Center in Freising. Determinations were done according to the methods of the European Commission due to regulation 159/2009. Amino acids were separated by ion chromatography and photometrically determined after Ninhydrin reaction by an amino acid analyzer (Biochrom 30). Samples of unfermented and fermented plant material were also sent to a specialized laboratory for determination of antinutritional substances. Trypsin inhibitory activity was determined enzymatic-colorimetrically by using benzoyl-DL-arginine-p-nitroanilide (BAPA) as a substrate (Kakade et al., 1969; Smith et al., 1980). Hydrolysable and condensed tannins were determined spectrophotometrically by the Folin-Denis reagent (Price and Butler 1977) and by vanillin hydrochloric acid (V-HCl) (Earp et al., 1981), respectively. Phytates (as phytic acid activity) were determined enzymatic-spectrophotometrically at 492 nm (Cat No. K-PHYT, Megazyme International Ireland Ltd., Wicklow, Ireland). Oxalates were determined by high-performance liquid chromatography (HPLC).

Statistical Analysis

To assess the separate and combined effect of the plant material (four species) and treatment (unfermented and fermented) on the nutritional composition (ash, protein and fibre content) of aquatic macrophytes a 4 x 2 factorial analysis of variance (ANOVA) was conducted. The means and standard error of means (SEM) for ash, protein and fibre content as a function of the two factors are presented in Table 1.5. The *F* test and Tukey's test for Post Hoc comparisons (P <0.05) were applied. All statistical analyses were performed using the SPSS (version 19) software package.

Results

Fermentation properties of selected aquatic plants (Trial I)

Table 1.1 shows the characteristics of the raw and lactic acid fermented aquatic macrophytes. Results indicated that dry matter content and water soluble carbohydrates (WSC) of all tested plants were very low, whereas crude fibre and ash content were relatively high. In addition, *Spirodela* and *Lemna* presented a high buffering capacity (BC). Thus, the fermentability coefficients of the raw aquatic macrophytes revealed a heavily fermentable material (FC<35).

Otherwise, the dry matter of the silages content ranged between desirable values (from 395 to 446 g kg^{-1}). The pH values ranged from 3.92 to 4.26 indicating good silages quality. Ammonia-N content was low and ranged from 5 to 58 g kg^{-1} in CP, except for the stems of *Eichornia* which reached a content of 164 g kg^{-1} ammonia-N in CP. Acetic acid content was also low in all samples. Silages were practically butyric and propionic acid-free. In contrast, concentrations of the lactic acid were high and ranged from 74.2 (stems of *Eichornia*) to 86.8 g kg^{-1} (leaves of *Eichornia*). DLG-quality label of the tested silages recorded the maximal mark.

Effect of fermentation on the nutritional quality of the aquatic plants (Trial II)

Table 1.2 shows the chemical composition of the tested aquatic macrophytes grown in rural zones of Colombia. The crude protein content ranged between 157 and 212 g kg^{-1}, except for stems of *Eichornia* with the lowest content (97.8 g kg^{-1}). Aquatic macrophytes have a relatively high crude fibre and ash content, and a low content of crude lipids. However, they are rich in minerals. Heavy metal concentrations of arsenic, selenium and mercury were not detectable, but cadmium and lead concentrations were detectable.

Table 1.3 shows the concentration of antinutritional substances in the raw and fermented aquatic macrophytes. Trypsin inhibitor, phytates (as phytic acid activity), soluble tannins and oxalate were detectable in all the raw aquatic plants, although the amounts did not exceed the tolerable limits for fish. Condensed tannins were not detectable in raw *Lemna* and *Azolla*, but in raw *Spirodela* and *Eichornia* leaves. In contrast, trypsin inhibitor and oxalates were significantly reduced by the fermentation process, as well as phytates, except for *Azolla*, which content of phytates did not change. Soluble and condensed tannins were not detectable in the fermented aquatic macrophytes.

Table 1.1: Chemical characteristics of raw and fermented aquatic macrophytes used in Trial I (expressed on dry matter basis).

Quality indicator	*Lemna minor*	*Spirodela polyrhiza*	*Azolla sp.*	*E. crassipes* (leaves)	*E.crassipes* (stems)
Factors affecting fermentation					
DM (g kg^{-1}), *fresh*	56.4	55.4	50.2	95.8	52.1
Ash (g kg^{-1})	153	195	64.0	132	215
Crude protein (g kg^{-1})	244	241	337	364	297
Crude fibre (g kg^{-1})	136	124	129	133	198
Crude fat (g kg^{-1})	31.0	22.0	31.0	32.0	14.0
Nitrate (g kg^{-1} NO_3)	2.45	1.30	0.22	17.7	60.0
BC[1] (g kg^{-1} Lactic ac.)	90.4	70.7	40715	36.3	22.7
WSC[2] (g kg^{-1})	<10.0	<10.0	<10.0	48.1	15.1
WSC/BC Quotient	0.11	0.14	0.46	1.33	0.66
FC[3]	6.53	6.67	8.73	20.2	10.5
DMmin[4] (g kg^{-1})	517	610	587	336	383
End-products of the lactic acid fermentation					
DM (g kg^{-1}), *fermented*	446	-	430	404	395
pH	4.04	-	3.92	4.24	4.26
Lactic acid (g kg^{-1})	85.7	-	80.0	86.8	74.2
Acetic acid (g kg^{-1})	4.30	-	5.20	4.90	2.80
Butyric acid (g kg^{-1})	0.00	-	0.60	0.00	0.00
Propionic acid (g kg^{-1})	0.00	-	0.00	0.00	0.00
Alcohols[4] (g kg^{-1})	0.60	-	2.90	0.40	0.00
Ammonia-N (g kg^{-1} total N)	2.70	-	0.30	1.10	9.50
Ammonia-N (g kg^{-1} CP)	58.0	-	5.00	16.0	164
DLG points	100	-	100	100	100

[1] BC= Buffer Capacity

[2] WSC= Water soluble carbohydrates

[3] FC= Fermentability coefficient

[4] Ethanol, methanol, n-propanol

Data corresponding to *Spirodela polyrhiza* were not analyzed

Table 1.2: Chemical composition of raw aquatic macrophytes used in Trial II (expressed on dry matter basis).

Chemical composition (g kg^{-1})	*Lemna minor*	*Spirodela polyrhiza*	*Azolla filiculoides*	*E.crassipes* (leaves)	*E.crassipes* (stems)
DM, *fresh dry*	952	930	910	929	923
Ash	270	241	258	167	251
Crude Protein	157	212	198	192	97.8
Crude Fat	22.5	22.5	17.1	33.0	25.5
Crude Fibre	167	172	159	156	223
NFE[1]	384	353	368	452	402
GE[2] (MJ kg^{-1})	11.4	12.2	11.9	13.9	10.4
Minerals (g kg^{-1})					
Ca	21.2	23.8	25.8	24.9	-
P	4,30	3.30	2.50	2.60	-
Na	17.0	14.5	22.8	3.50	-
K	25.5	22.2	15.2	24.6	-
Mg	17.0	15.1	22.9	4.10	-
Zn (mg kg^{-1})	9.64	11.3	160.7	43.1	-
Cu (mg kg^{-1})	4.34	8.81	14.81	7.14	-
Cr (mg kg^{-1})	1.56	2.81	18.19	3.69	-
Al (mg kg^{-1})	33.9	28.8	1.50	51.4	-
Heavy metals (mg kg^{-1})					
Cd	0.48	0.76	1.31	0.53	-
Ar	N.d.	N.d.	N.d.	N.d.	-
Se	N.d.	N.d.	N.d.	N.d.	-
Hg	N.d.	N.d.	N.d.	N.d.	-
Pb	3.14	3.23	3.98	3.44	-

N.d.: no detectable

[1] Nitrogen-free Extract (NFE) = 100-(Ash+ Protein+ Fat + Fibre)

[2]Gross Energy (GE) calculated by 23.9 MJ kg^{-1} protein; 39.8 MJ.kg^{-1} fat and 17.6 MJ.kg^{-1} (NFE+CF)

Table 1.3: Concentration of anti-nutritional substances in raw and fermented aquatic macrophytes (Trial II).

Anti-nutritional substances	*Lemna minor*		*Spirodela polyrhiza*		*A. filiculoides*		*E. crassipes* (leaves)	
	Raw	Fermented	Raw	Fermented	Raw	Fermented	Raw	Fermented
Trypsin inhibitor (mg.g^{-1})	2.31	0.50	0.80	0.17	1.86	1.37	1.05	0.60
Phytates (% phytic ac.)	0.32	0.12	0.25	0.11	0.15	0.15	0.15	0.12
Soluble tannins (%)	0.30	N.d.	1.31	N.d.	0.44	N.d.	0.70	N.d.
Condensed tannins (%)	N.d.	N.d.	3.87	N.d.	N.d.	N.d.	0.99	N.d.
Oxalates (%)	2.02	0.04	0.10	N.d.	1.67	0.19	0.52	0.10

N.d.: not detectable

The amino acid profile of tested local macrophytes and amino acids requirements of the tropical fish Nile tilapia (*Oreochromis niloticus*) and Pacu (*Piaractus mesopotamicus*) are presented in Table 1.4. The protein of the raw material resulted in a similar amino acid profile among plants and contained 5.30 to 6.28 g/100g lysine and 1.72 to 2.04 g/100 g methionine in the dietary protein. Interesting, the tested aquatic macrophytes showed to be rich in aspartic acid and glutamic acid.

Table 1.4: Amino acids profile of the aquatic macrophytes harvested from water bodies at northern Colombia (Trial II) and amino acids requirements (g $100g^{-1}$ Dietary Protein) of common cultured tropical fish.

Amino acids (g $100g^{-1}$ Protein)	*Lemna minor*	*Spirodela polyrhiza*	*Azolla filiculoides*	*E. crassipes* (Leaves)	AA requirement	
					Tilapia[1]	Pacu[2]
			Essential			
Arginine	5.79	6.25	6.16	6.06	4.20	3.19
Histidine	1.72	1.92	1.96	2.31	1.72	1.14
Isoleucine	4.93	5.05	5.07	5.07	3.11	2.09
Leucine	9.36	9.17	9.27	9.48	3.39	4.12
Lysine	5.30	5.90	5.28	6.28	5.12	1.51
Methionine	1.72	1.92	1.76	2.04	3.21	1.20
Phenylalanine	5.54	5.47	5.62	6.12	5.59	2.06
Threonine	4.93	4.69	5.07	4.79	3.75	2.07
Valine	6.65	6.47	6.43	6.17	2.80	2.05
Tryptophan	1.60	1.42	1.62	2.04	1.00	-
			Non-essential			
Alanine	7.64	7.04	6.83	6.61	-	-
Aspartic acid	10.2	9.88	10.4	10.4	-	-
Cystine	1.48	1.21	1.01	1.05	-	0.37
Glutamic acid	13.3	12.5	12.9	12.4	-	-
Glycine	6.65	6.47	6.16	5.73	-	-
Proline	5.17	5.40	5.14	5.18	-	-
Serine	4.56	4.83	5.01	4.13	-	-
Tyrosine	3.45	4.41	4.33	4.13	-	1.72

[1]Nile Tilapia (*Oreochromis niloticus*), Santiago and Lovell (1988).

[2]Pacu (*Piaractus mesopotamicus*), Bicudo et al. (2009).

The nutritional composition of the unfermented and fermented macrophytes is presented in Table 1.5. All tested variables showed significant differences at both factors except for ash content between treatments. The interaction between the plant species and treatments was significant and was not always in the same direction (*disordinal* interaction). Whereas crude fibre was significantly reduced ($P < 0.05$) in all fermented plants, crude protein varied among the plants species and resulted significantly higher ($P < 0.05$) in fermented *Lemna* and

Spirodela and significantly lower (P < 0.05) in fermented *Azolla* and *Eichornia* when compared to the respectively unfermented plant material. Ash content did not change.

Table 1.5: Nutrient composition (g kg^{-1}) of unfermented and fermented aquatic macrophytes (Trial II).

Experimental material	Parameters		
	Ash	Protein	Fibre
Unfermented Lemna	215.03	111.67	129.62
Unfermented Spirodela	330.05	177.14	141.33
Unfermented Azolla	298.48	189.48	117.60
Unfermented Eichornia	172.89	218.06	147.25
Fermented Lemna	210.23	131.01	126.07
Fermented Spirodela	321.74	184.19	104.44
Fermented Azolla	339.41	163.14	102.47
Fermented Eichornia	165.89	210.52	142.63
Plants (P)		Means	
Lemna	212.63^{ab}	121.34^{a}	127.84^{a}
Spirodela	325.90^{a}	180.67^{b}	122.88^{b}
Azolla	318.94^{b}	176.31^{c}	110.04^{c}
Eichornia	169.39^{ab}	214.29^{d}	144.94^{d}
SEM	3.99	0.72	1.06
Prob.	0.001	0.001	0.001
Treatment (T)			
Unfermented	254.11^{a}	174.09^{a}	133.95^{a}
Fermented	259.32^{a}	172.22^{b}	118.90^{b}
SEM	2.82	0.51	0.75
Prob.	0.211	0.019	0.001
Interaction P x T			
Prob.	0.001	0.001	0.001
Statistics		Variables F values	
Plants (P)	381 *	2866 *	184 *
Treatment (T)	1.70^{NS}	6.79 *	200 *
Interaction P x T	8.92 *	187 *	52.9 *

^{NS}= Not significant, * significant (P<0.05)

^{abcd} Means in same column without common superscript are different at P<0.05

Discussion

Fermentation properties of the selected aquatic plants (Trial I)

The raw aquatic macrophytes presented a very low dry matter content (DM <100 gkg^{-1}), a low sugar content (WSC<50 g kg^{-1} DM), and a relatively high buffering capacity. Therefore they showed very low WSC/BC ratios and consequently low fermentation coefficients (FC<35) indicating that they are difficult to ferment. According to Pahlow et al. (2003) a WSC of 75 g kg^{-1} DM is the lowest threshold to establish a good fermentation. Likewise, enough amounts of natural populations of LAB are required. However, they are often low in number and hetero-fermentative on the plant material, so that they produce end-products other than lactic acid (Ennahar et al., 2003). Considering that mentioned, the addition of molasses as source of WSC and the inclusion of a LAB inoculant are required to improve the silage potential of the tested aquatic macrophytes.

In fact, the amounts of fermentation end-products in the aquatic macrophytes silages were comparable to those reported for common silages as grass, corn, and alfalfa. In general, the pH values ranged lower than 4.5 as recommended by Kaiser et al. (2006), and lactic acid concentrations were closely related to those reported by Kung (2001) for good quality alfalfa silages (70 – 80 g kg^{-1} lactic ac.) and grass silages (60 - 100 g kg^{-1} lactic ac.). The DLG points obtained in this study indicated very good silage fermentation.

Effect of fermentation on the nutritional quality of the aquatic plants (Trial II)

Characteristics of the raw aquatic macrophytes

The nutritional composition of the raw aquatic macrophytes in Trial II was characterized by a high ash and crude fibre content, a limited lipid content (17-33 g kg^{-1}), an acceptable protein content (160-210 g kg^{-1} DM) considering the requirement of fish, and a good balanced amino acid composition. The content of ash and fibre for the tested plants in this study was comparable with values reported for several aquatic plants by Bairagi et al. (2002), El-Sayed (2003), Kalita et al. (2007), and Leterme et al. (2009). Low content of lipids was also reported by Bairagi et al. (2002) for *Lemna polyrhiza* (15 g kg^{-1}) as well as by El-Sayed (2003) for *Eichornia crassipes* (10 g kg^{-1}). In contrast, Kalita et al. (2007) reported a higher content of lipids for *Lemna minor* (50 g kg^{-1}) from northeast India when compared to this study. The deficit of lipids should be supplemented by additional components when the aquatic macrophytes are included into fish diets.

Aquatic macrophytes are rich in minerals, exceeding the fish requirements but not the critical values for fish. Mineral composition of the raw aquatic macrophytes revealed a comparable content among plants, except for *Azolla*. Concentrations of calcium and phosphorus were nearly similar in all the plants. Calcium and potassium were the most abundant minerals tested. Phosphorus content in *Lemna* and *Spirodela* ranged within the requirements reported for common finfish (NRC 1993), whereas the Ca:P ratio was higher than the requirement for fish. The reduction of calcium in the mineral mixture used in formulated diets must be considered.

Azolla showed the highest amount of zinc (161 mg kg^{-1}), copper (14.8 mg kg^{-1}) and chromium (18.2 mg kg^{-1}). Although the requirement of zinc for the majority of fish is much lower, varying from 15 to 30 mg/kg (NRC 1993), higher levels of supplemental zinc are frequently included in the practical diets to compensate the reduced zinc bioavailability caused by other dietary factors such as phytates. In some fish species (trout and carp) the tolerable limit of zinc in diets has been reported as 1900 mg kg^{-1} (Jeng and Sun 1981; NRC 1993). Likewise, the requirement of copper for fish, which varies from 3 to 5 mg kg^{-1}, is much lower than in the fern *Azolla*. However, it does not exceed the tolerable limit for fish diets, which has been reported as 150 mg kg^{-1} (NRC 1993). Mineral concentration should be considered before the inclusion of *Azolla* into fish diets depending on the fish species and its particular tolerable limits.

The heavy metal concentration is particularly important as aquatic plants tend to accumulate them. In this study the concentrations of the heavy metals arsenic, selenium and mercury were not detectable. Cadmium ranged in very low amounts (0.48-1.31 mg kg^{-1}), whereas lead ranged from 3.14 to 3.98 mg kg^{-1}. Cadmium has been reported to be absorbed through the gastrointestinal tract of fish (NRC 1993) and causes liver necrosis and mortality at doses of 5 mg kg^{-1} of body weight. In contrast, Hodson et al. (1978) reported that dietary lead was not absorbed by rainbow trout fed lead in different amounts. In this study the concentrations of heavy metals were not critical, but if aquatic macrophytes were used as exclusive feed source for fish, heavy metal contamination of feed, particularly cadmium retention, must be considered since it reduces fish growth, feed conversion and can be toxic.

Characteristics of the fermented aquatic macrophytes

Except for phytates content in *Azolla,* the antinutritional substances, trypsin inhibitor, phytates, tannins (hydrolyzed and condensed), and oxalates were significantly reduced by the lactic acid fermentation. According to Francis et al. (2001) the tolerable limit of trypsin inhibitor is below 5 g kg^{-1} TI for the most cultured fish. Likewise, phytates (as phytic acid) and sodium phytates have been reported to cause depression in growth and food conversion efficiency of fish at levels of 5 g kg^{-1} and 10 g kg^{-1} in diets (Spinelli et al., 1983, Hossain and Jauncey 1993). The level of trypsin inhibitor (lower than 1.37 mg g^{-1} TI), phytates (lower than 1.5 g kg^{-1} phytic ac.), tannins (not detectable) and oxalate (about zero) in fermented aquatic macrophytes did not exceed the critical value for commonly cultured fish.

Crude fibre was significantly lower in the fermented aquatic macrophytes when compared to the unfermented samples. The decrease in the fibre content may be due to partial acid hydrolysis of hemicelluloses as a result of microbial utilization involving fermentation (Jones, 1975). Crude protein content was also affected by fermentation. However, the effects of lactic acid fermentation on the protein content are conditional and strongly depend on the plant species. Thus, changes can be explained by differences in the chemical properties of the plant species, by the effect of molasses supplementation, and by the processes occurred over the six weeks of fermentation on the plant material. The decreases of crude protein in fermented *Azolla* and *Eichornia* may be related to a slower acidification on the silage leading to an increase in proteolysis (Cussen et al., 1995), whereas the increases of crude protein in fermented *Lemna* and *Spirodela* may have been occurred through microbial synthesis (Wee, 1991).

In general, lactic acid fermentation is highly recommendable before the inclusion of aquatic macrophytes into fish diets as high fibre content of plant ingredients has a negative impact on digestibility (De Silva et al., 1990). The raw aquatic macrophytes would not be recommended as exclusive nutrient sources. But aquatic macrophytes silages may be used for partial replacement of protein sources in practical fish diets or as mineral source for the supplementation of basic fish feed in farming.

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CHAPTER 2.

Digestibility coefficients of sun dried and fermented aquatic macrophytes for Cachama blanca, *Piaractus brachypomus* (Cuvier, 1818)

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Abstract

The apparent digestibility coefficients of dry matter ($ADC_{drymatter}$), crude protein ($ADC_{protein}$) and the gross energy (ADC_{energy}) of the aquatic macrophytes *Spirodela polyrhiza, Lemna minor* and *Azolla filiculoides* treated by sun drying and lactic acid fermentation were determined for juveniles (31.0 ± 5.2 g) of Cachama blanca, *Piaractus brachypomus*. Each test ingredient was included at 300 g kg^{-1} in a semipurified reference diet containing chromium oxide as an indicator. Faeces were collected by a specific sedimentation system. The $ADC_{drymatter}$, $ADC_{protein}$ and ADC_{energy} of the reference diet were 56.8%, 97.2% and 70.1%, respectively. The 3 x 2 factorial analysis of variances indicated that the digestibility coefficients of the tested macrophytes were significantly different between plant material and treatments, but not between their combinations. $ADC_{protein}$ ranged from 74.9% to 84.5% in fermented macrophytes and from 51.1% to 60.4% in sundried macrophytes, indicating that the fermentation process had increased the protein digestibility of the aquatic plants ($P<0.05$). Among the plants, fermented *Spirodela polyrhiza* showed the highest nutrient and energy digestibility. Lactic acid fermentation is a highly recommendable treatment when aquatic macrophytes should be include into diets for Cachama blanca.

Keywords: *Cachama, protein digestiblity, energy digestibility, fermentation*

Resumen

Los coeficientes de digestibilidad aparente de la materia seca ($ADC_{materia\ seca}$), la proteína cruda ($ADC_{proteína\ cruda}$) y la energía bruta ($ADC_{energía\ bruta}$) de las macrófitas acuáticas *Spirodela polyrhiza, Lemna minor* y *Azolla filiculoides*, tratadas mediante secado al sol y fermentación ácido láctica, fueron determinados para juveniles de Cachama blanca, *Piaractus brachypomus* (31.0 ± 5.2 g). Cada ingrediente de ensayo se incluyó en 300 g kg^{-1} en una dieta de referencia semipurificada que contenía óxido de cromo como indicador. Las heces se recogieron mediante un sistema de sedimentación específico. Los $ADC_{materia\ seca}$, $ADC_{proteína\ cruda}$ y $ADC_{energía\ bruta}$ de la dieta de referencia fueron 56.8%, 97.2% y 70.1%, respectivamente. El análisis de varianzas factorial 3 x 2 indicó que los coeficientes de digestibilidad de las macrófitas estudiadas variaron significativamente entre el material vegetal y los tratamientos, pero no entre sus combinaciones. $ADC_{proteína\ cruda}$ varió de 74.9% a 84.5% en los macrófitas fermentadas y de 51.1% a 60.4% en los macrófitas secados al sol, lo que indica que el proceso de fermentación incrementó la digestibilidad de la proteína disponible en las plantas acuáticas ($P<0.05$). Entre las plantas estudiadas, la *Spirodela polyrhiza* fermentada mostró la mayor

digestibilidad de los nutrientes y la energía. La fermentación ácido láctica es así un tratamiento altamente recomendable cuando se incluyen macrófitas acuáticas en las dietas para Cachama blanca.

Palabras clave: *Cachama, digestibilidad de la proteína, digestibilidad de la energía, fermentación*

Resumo

Os coeficientes de digestibilidade aparente da matéria seca ($ADC_{matéria\ seca}$), a proteína bruta ($ADC_{proteína\ bruta}$) e energia bruta ($ADC_{energia\ bruta}$) de macrófitas aquáticas *Spirodela polyrhiza, Lemna minor* e *Azolla filiculoides* tratado por secagem ao sol e fermentação de ácido lático foram determinados para juvenil da Pirapitinga (31.0 ± 5.2 g). Cada ingrediente teste foi incluído a 300 g kg-1 em uma dieta de referência semipurificada contendo óxido de crómio como um indicador. As fezes foram coletadas através de um sistema de sedimentação específico. A $ADC_{matéria\ seca}$, $ADC_{proteína\ bruta}$ e $ADC_{energia\ bruta}$ da dieta referência foram 56.8%, 97.2% e 70.1%, respectivamente. A análise fatorial de variância 3 x 2 indicaram que os coeficientes de digestibilidade das macrófitas estudadas variou significativamente entre os materiais e tratamentos de planta, mas não entre as suas combinações. A $ADC_{proteína\ bruta}$ variou de 74.9% a 84.5% em macrófitas fermentadas e de 51.1% para 60.4% em macrófitas desidratadas, indicando que o processo de fermentação aumentou a digestibilidade de proteína disponíveis nas plantas aquáticas ($P < 0.05$). Entre as plantas estudadas, a Spirodela polyrhiza fermentada apresentou a maior digestibilidade dos nutrientes e energia. Fermentação de ácido láctico é, portanto, um tratamento altamente recomendado quando macrófitas aquáticas estão incluídas em dietas para a Pirapitinga.

Palavras-chave: *Pirapitinga, digestibilidade da proteína, digestibilidade da energia, fermentação*

Introduction

In feed formulation, the most important characteristics of the components are their nutrient quantity and quality as well as their digestibility, mainly digestible protein and energy (Austreng 1978; Fagbenro 1996). The apparent digestibility coefficients (ADCs) provide valuable information for the formulation of nutritional and economically feasible diets. Particularly, diet and dietary compound digestibility are essential for exact determination of nutrient demands in fish metabolism. In addition, ensuring a high diet digestibility preserves the aquatic environment by avoiding the accumulation of indigestible ingredients.

For the Amazonian fish *Piaractus brachypomus* as for the majority of characids, the scarce information on nutritional demand existent refers mainly to ingredients which are frequently used in common commercial aquafeeds, namely fish meal, soybean or soybean-derived products and wheat products (Quintero *et al.* 1993; Hernández 1993; Torres *&* Uribe 1995; Fernandes *et al.* 2004; Gutierrez-Espinoza *&* Vásquez-Torres 2008). Considering increased prices of aquafeeds, the identification of less expensive and locally available feed sources has become necessary to assure the proposed aquaculture development, particularly in rural areas. In fact, the use of non-conventional feed sources is an important key to reduce feed costs in fish cultivation and therefore to ensure small-scale farmers' income in the marginal areas of the Neotropics.
Aquatic macrophytes are one of the most abundant plant materials in the Neotropical floodplain systems and a natural feed source for some native fish species. Although, they have been widely used as a nutrient source in diets for tropical freshwater fish, principally tilapia and carp (Edwards 1980; Ray *&* Das 1992; Bairagi *et al.* 2002; El-Sayed 2003; Henry-Silva *et al.* 2006), information on their nutritional value for *Piaractus brachypomus* is lacking.

In spite of the great nutritional potential of aquatic macrophytes, their utilization in native forms as fish feed remains low due to their relative high fibre and ash content as well as the presence of trypsin inhibitors, phytates, tannins and oxalates, among other antinutritional substances, which reduce their digestibility. To enhance the nutritional value of plant materials for fish, simple methods for processing suitable for marginal areas have been investigated. Thus, the fermentation process of aquatic macrophytes seems to be adequate, since it considerably reduces fibre content and antinutritional substances present in the plants (Bairagi*et al.* 2002, El-Sayed 2003, Cruz *et al.* 2011).

The South American fish Cachama blanca or Pirapitinga (common names in Colombia and Brazil, respectively) *Piaractus brachypomus* (Characidae), is an omnivorous fish with a predominantly herbivorous-feeding behaviour (Silva 1985). Its natural diet consists principally of plant products and in a lower amount of fish and crustacean. It is widely found in the Amazon and Orinoco river basins (Jégu 2003) and one of the most important native fish species in Colombia. Moreover, it is the primary fish species in the Colombian national program of food safety and occupies the second place in the Colombian national aquaculture production (Espinal *et al.* 2005).

Considering that processed aquatic macrophytes may be a suitable source of plant protein for the small-scale characid production in Colombia, the present study was conducted to determine, dry matter, crude protein and gross energy digestibility of selected aquatic macrophytes (*Spirodela polyrhiza, Lemna minor* and *Azolla filiculoides*) treated by sun drying and lactic acid fermentation methods for juveniles of *Piaractus brachypomus*.

Material and Methods

Sun Drying and Fermentation of Aquatic Plants

The aquatic macrophytes used for experimental diets were harvested as wild or uncultivated plant material from water bodies in northern Colombia. Plants were collected continuously during a period of 12 weeks. After taxonomical identification, the plant material was cleaned and divided in two parts. The first part was sundried until its dry weight was constant. The second part was fermented. In order to obtain the required DM content for silages, which is approximately 300-400 g kg^{-1} for grass and forages (DLG 2006), the freshly harvested aquatic plants were mixed with wilted aquatic plants of the same sample. Afterwards, these mixtures were fermented.

The lactic acid silage fermentation process was carried out using a commercial silage lactic acid bacteria (LAB) inoculant as described by Cruz *et al.* (2011) following to the methodology proposed by Johnson *et al*. (2005). Finally, the silages were opened and dried in an oven at 45 °C for 48 h before their inclusion into the experimental diets.

Experimental Diets

The six experimental diets were composed of 700 g kg^{-1} of a reference diet based on a semipurified diet performed by Vásquez-Torres *et al.* (2002) and 300 g kg^{-1} of each tested ingredients: *Spirodela polyrhiza* (Giant Duckweed), *Lemna minor* (Duckweed) and *Azolla filiculoides* (Fern Azolla) treated by sun drying or lactic acid fermentation (Table 2.1).

Chromic oxide (Cr_2O_3) at an inclusion level of 5 g kg^{-1} was used as external inert marker. Diets were finely ground, mixed and pelleted to sizes of 4 mm.

Animals and Feeding Trial

A total of 630 juveniles of *Piaractus brachypomus* with a similar initial body weight of 31.0 ± 5.2 g (mean ± SD) were randomly stocked in 21 experimental 250L-tanks. The experiment was conducted in a closed recirculation-system with constant aeration and a photoperiod of approximately 12 h of light. The water quality was monitored daily in the morning with respect to temperature, pH, and dissolved oxygen. Prior to the experiment, the fish were fed with the reference diet twice daily and for a period of two weeks in order to accustom them to the experimental conditions. Afterwards, seven experimental diets (one reference diet and six test diets) were randomly assigned to triplicate experimental groups. Fish were manually fed the experimental diets once daily to apparent satiety.

For faeces collection nine cylindrical-conical tanks (200 L) equipped with a terminal faeces removal container system as described by Vásquez-Torres et al. (2011a) were used. Stocked fish of each experimental tank were transferred to the cylindrical-conical tanks after feeding. Faeces collection was done according to a specific schedule. The samples were collected at 60 min intervals over a period of 12 h per day during two wks. The faeces samples removed from the containers were subsequently rinsed in distilled water, oven dried at 60 °C for 72 h and stored until laboratory analysis.

Table 2.1. Composition of the reference and test diets evaluated for *Piaractus brachypomus*.

Ingredients (g kg^{-1})	Reference Diet (Ref)	Test Diet
Caseine	354	248
Gelatine	43	30
Dextrine	242	169
α-Cellulose	180	126
Carboximetil Cellulose	68	48
Fishoil	33	23
Sunfloweroil	33	23
Vitamin premix[1]	2	1
Microminerals premix[2]	1	1
Macrominerals premix[3]	38	27
Ascorbicacid (Stay C-35)	1	1
Chromiumoxide (Cr_2O_3)	5	4
Test Ingredient	0	300

[1]Rovimix vitamin: ®Lab. Roche S.A. 0.5 (Vit A $8.0*10^6$ UI, Vit D3, $1.8*10^6$ UI, Vit E 66.66 g, Vit B1 6.66 g, Vit B2 13.33 g, Vit B6 6.66 g, Calcium pantothenic 33.33 g, Biotin 533.3 mg, Folic acid 2.66 g, Ascorbic acid 400.0 g, Nicotinic acid 100.0 g, Vit B12 20.0 mg, Vit K3 6.66 g, csp vehicle 1.0 kg.

[2]Micro-minerals premix: ®Lab. Roche S.A. 1.0 (Composition per 100 g the product: Mg 1.0, Zn 16.0, Fe 4.0, Cu 1.0, I 0.5, Se 0.05, Co 0.01).

[3]Macro-minerals premix: 4.02 (Composition g 100 g^{-1} the product: Ca (H_2PO_4) 13.6 g, Calcium lactate 34.85 g, $2MgSO_4.7H_2O$ 13.2 g, KH_2PO_4 24 g, NaCl 4.5 g, $AlCl_3$ 0.015 g, CMC 9.835 g).

Chemical Analysis

The nutrient composition of the sundried and fermented material, as well as the diets and the faecal samples collected from each tank were determined in triplicates and performed following the AOAC (1990) procedures. The energy content as gross energy was determined by using an adiabatic bomb calorimeter (PARR 121 EA, USA).

Calculations

The amount of inert marker (Cr_2O_3) contained in the diets and the faecal samples were determined by wet-acid digestion method (Furukawa *&* Tsukahara 1966). The ADCs for the nutrients and energy of the reference and test diets were calculated using the formula described by Nose (1960), as follows:

$$ADC_{diet} = 100 - \left\lfloor 100 \times (\%Cr_2O_{3diet} / \%Cr_2O_{3faeces}) x (\%Nut_{faeces} / \%Nut_{diet}) \right\rfloor$$

Where:

$ADC_{diet} =$ Apparent digestibility coefficient of the nutrients or energy in diets

$\%Cr_2O_{3diet} =$ % of chromium content in diets

$\%Cr_2O_{3faeces} =$ % of chromium content in faeces

$\%Nut_{diet} =$ % of nutrient or energy in diets

$\%Nut_{faeces} =$ % of nutrient or energy in faeces

ADCs of the test ingredients were calculated based on the digestibility of the reference diet and test diets using the formula suggested by Bureau *et al.* (1999), which considers the relative nutrient contribution from the basal diet and the test single ingredients (Bureau andHua 2006).

$$ADC_{ing} = ADC_{comdiet} + \left\lfloor (ADC_{comdiet} - ADC_{refdiet}) \times (0.7 \times \%Nut_{refdiet} / 0.3 \times \%Nut_{ing}) \right\rfloor,$$

Where:

$ADC_{ing} =$ Apparent digestibility coefficient of the nutrients or energy in test ingredients

$ADC_{testdiet} =$ Apparent digestibility coefficient of the nutrients or energy in test diets

$ADC_{refdiet}$ = Apparent digestibility coefficient of the nutrients or energy in reference diet

$\%Nut_{refdiet}$ = % of nutrient or energy in reference diet

$\%Nut_{ing}$ = % of nutrient or energy in ingredients

Digestible protein (DP) and digestible energy (DE) were calculated using the data corresponding to protein and energy digestibility, crude protein (CP) and gross energy (GE) content of test ingredients.

$$DP_{ing} = (\%CP_{ing} x ADC_{ing})/100$$

$$DE_{ing} = ((kJ/g)GE_{ing} x ADC_{ing})/100$$

Statistical Analysis

In order to assess the separate and combined effect of the plant material (three species) and treatment (sundried and fermentation) on the dry matter, protein and energy digestibility in Cachama blanca a 3 x 2 factorial analysis of variances (ANOVA) was conducted. The means and standard error of means (SEM) for dry matter, protein and energy digestibility as a function of the two factors are presented in Table 1.3.The *F* test and Tukey's test for Post Hoc comparisons ($P < 0.05$) were applied. Homogeneity of the variances was determined by Levene's Test and by Dunnett's T3 Test ($P < 0.05$) for homogeneous and inhomogeneous variances, respectively. All statistical analysis was carried out using the SPSS (version 19) software package.

Results

The water quality parameters presented during the experimental period were within optimal requirements for *Piaractus brachypomus,* reported by Vasquez-Torres (2005): temperature 25.6 ± 0.99 ºC; pH 6.42 ± 0.16; and dissolved oxygen 5.28 ± 1.45 mg L^{-1}. All diets were satisfactorily consumed, indicating that no palatability problem was encountered with any of the treatments during the experiment.

The proximate composition of the test ingredients, reference diet and test diets (Table 2.2) indicates that the reference diet had the lowest ash (38.5 g kg^{-1}) and crude fibre (58.5 g kg^{-1})

content, and the highest crude protein (342.1 g kg^{-1}) and energy (19.7 kJ g^{-1}) content. Likewise, among test diets those containing fermented plants showed the lowest crude fibre and ash content, whereas protein and lipids contents were slightly higher compared to diets containing sundried macrophytes. The dietary gross energy was not different in the test diets.

Table 2.2. Chemical composition (g kg^{-1}) and gross energy (kJ g^{-1}) of the ingredients, reference and test diets in the digestibility trials. Values (mean ± SD) are expressed on a dry matter basis.

	Ash	Crude protein	Crude lipids	Crude Fibre	NFE[1]	Gross Energy
Ingredients						
FermentedSpirodela(FS)	241.9	229.7	38.4	76.3	413.8	14.9
FermentedLemna(FL)	246.3	227.9	37.3	69.9	418.6	14.6
FermentedAzolla(FA)	205.1	224.3	34.8	101.6	434.2	13.7
SundriedSpirodela(SS)	297.8	149.3	23.8	142.4	386.7	14.1
SundriedLemna(SL)	375.5	135.7	12.7	141.5	334.6	14.1
SundriedAzolla(SA)	343.3	145.8	13.6	142.0	355.2	13.3
Test diets						
Reference Diet (Ref)	38.5	342.1	21.8	58.5	539.1	19.7
Ref+FS	90.3	310.5	31.7	63.9	503.7	17.9
Ref+FL	91.8	312.7	31.4	61.9	502.1	17.9
Ref+FA	79.1	319.7	34.3	71.4	495.5	17.5
Ref+SS	116.7	283.4	22.5	84.5	492.9	17.6
Ref+SL	141.5	279.4	19.0	85.5	474.8	17.5
Ref+SA	130.8	283.9	18.6	85.3	481.4	17.4

[1] Nitrogen-free Extract (NFE) = 100 - (Ash + Protein + Fibre + Fat)

The $ADC_{drymatter}$, $ADC_{protein}$ and ADC_{energy} of the tested ingredients were significantly different between plant material types and processing methods, but not between their combinations (Table 2.3). However, values tended to be higher for the fermented aquatic plants indicating the major effect of the processing method on the digestibility. Among test ingredients, the highest $ADC_{drymatter}$, $ADC_{protein}$ and ADC_{energy} were obtained for fermented macrophytes, whereas the significantly lowest value was displayed by the sundried macrophytes. Fermented *Spirodela polyrhiza* showed the highest $ADC_{drymatter}$ (84.6 %), $ADC_{protein}$ (84.5%) and ADC_{energy} (62.6 %), whereas sundried *Azolla filiculoides* showed the lowest $ADC_{drymatter}$ (43.8 %), $ADC_{protein}$ (51.1 %) and ADC_{energy} (37.8 %).

Table 2.3. Apparent digestibility coefficients of dry matter, crude protein (CP) and gross energy (GE) of the test ingredients (mean ± SD, n = 3).

	ADC dry matter (%)	ADC crude protein (%)	ADC gross energy (%)	Digestible CP (g kg^{-1})	Digestible GE (Kj/g)
Test Ingredients			Mean ± SD		
FermentedSpirodela	84.6 ± 6.7	84.5 ± 2.4	62.6 ± 8.7	194 ± 5.9	9.3 ± 1.2
FermentedLemna	68.1 ± 3.6	77.8 ± 1.7	56.6 ± 3.5	177 ± 5.6	8.2 ± 0.7
FermentedAzolla	65.8 ± 4.5	74.6 ± 2.1	47.2 ± 5.9	167 ± 3.6	6.4 ± 0.8
SundriedSpirodela	61.3 ± 3.6	60.4 ± 1.2	51.1 ± 3.2	90.1 ± 0.8	7.1 ± 0.4
SundriedLemna	56.6 ± 7.7	55.4 ± 4.3	48.4 ± 5.1	75.1 ± 6.1	6.8 ± 0.6
SundriedAzolla	43.8 ± 2.3	51.1 ± 1.4	37.8 ± 2.5	74.5 ± 4.5	5.0 ± 0.4
Plants (P)			Mean values		
Spirodela	69.93^{a}	72.42^{a}	56.82^{a}	142.08^{a}	8.20^{a}
Lemna	62.37^{ab}	66.58^{b}	52.52^{a}	126.20^{b}	7.52^{a}
Azolla	54.8^{b}	62.85^{c}	42.47^{b}	120.96^{b}	5.72^{b}
SEM	2.078	0.977	2.144	1.954	0.289
Treatment (T)			Mean values		
Lacticacid ferm.	70.8^{a}	79.0^{a}	55.4^{a}	179.6^{a}	7.98^{a}
Sun drying	53.9^{b}	55.6^{b}	45.7^{b}	79.9^{b}	6.31^{b}
SEM	1.697	0.798	1.750	1.595	0.236
Statistics			F values		
Plants (P)	13.26 *	24.33 *	11.80 *	31.67 *	19.66 *
Treatment (T)	49.79 *	428.59 *	15.39 *	1951.71 *	24.89 *
Interaction PxT	1.631^{NS}	0.190^{NS}	0.149^{NS}	2.281^{NS}	0.488^{NS}

^{NS} = Not significant, * = Significant (P<0.05)

^{abc} Means in same column without common superscript are different at P <0.05

Discussion

High ash content negatively influence the digestibility of plant materials. The lower $ADC_{drymatter}$ of sundried aquatic macrophytes could be explained by their high mineral content (ash content) when compared to the fermented aquatic plants. Edwards (1980) reported that it reduces the nutritional value of aquatic macrophytes and it is considered as the main reason why animals refuse to eat them in large quantities. The lower $ADC_{protein}$ of sundried plants may be related to their high fibre content, which ranged closely to 140 g kg^{-1}, whereas in fermented plants the fibre content ranged between 69.9 and 101.6 g kg^{-1}. Similar results were reported by Fernandes *et al*. (2004) in diets for *Piaractus brachypomus*, who attributed the low $ADC_{protein}$ of wheat bran (61.6%) to the high crude fibre content of wheat products (about 100 g kg^{-1} according to NRC 1993) as well as to its high level of phytates (8.8 g kg^{-1}). In general, the fibre content as well as the ash content was strongly diminished in diets containing fermented plants.

The $ADC_{protein}$ of fermented aquatic macrophytes (between 74.6 and 84.5%) may be compared to those reported for ingredients commonly used in diets for *Piaractus brachypomus*. The $ADC_{protein}$ of toasted whole soybean (81.1%), soy cake (83.2%) (Gutiérrez-Espinosa & Vásquez-Torres 2008), wheat bran (82.9%), corn gluten (84.3%), wheat bran (82.9%) (Vásquez-Torres *et al.* 2007), raw whole soybean (75.6%) and corn (85.1%) (Fernandes *et al.* 2004) were nearly related to those obtained for fermented aquatic plants in the present study, particularly for fermented *Spirodela*. Lower $ADC_{protein}$ were found in wheat bran (61.6%) (Fernandes *et al.* 2004) and Colombian fish meal (68.5%) (Vásquez-Torres *et al.* 2007), whereas higher $ADC_{protein}$ were reported by Fernandes *et al.* (2004) for Brazilian fish meal (90.5%) and by Vásquez-Torres *et al.* (2007) for palmiste (87.9%), yellow corn (87.2%) and sunflower cake (86.3%).
In another study with Pacu (*Piaractus mesopotamicus*), a closely-related species to *Piaractus brachypomus*, Abimorad*et al.* (2007) reported similar $ADC_{protein}$ for yeast (81.5%), Brazilian fish meal (84.6%) and corn (85.8%), whereas $ADC_{protein}$ obtained for wheat bran (87.7%), soybean meal (90.6%) and corn gluten meal (95.6%) were higher compared to those reported in the present study for fermented *Spirodela polyrhiza*.

As a consequence of their higher $ADC_{protein}$, fermented macrophytes showed a significantly higher digestible protein content (from 167 g kg^{-1} to 194 g kg^{-1}) than sundried macrophytes (from 74.5 g kg^{-1} to 90.1 g kg^{-1}). These results are also consistent to those previously reported for other warm water fish by several authors. In earlier studies with Indian major carp, Ray & Das (1992) suggested that fermented macrophytes had a higher protein digestibility in relation to sundried macrophytes. More recently, Bairagi*et al.* (2002) evaluated raw and fermented *Lemna polyrhiza* leaf meal in formulated diets for a fish of the carp family, Rohu (*Labeorohita*), and reported that the $ADC_{protein}$ for raw material meal was much lower at all levels of inclusion in comparison to the those obtained for the fermented meal. Also, El-Sayed (2003) demonstrated that fermentation of *Eichornia crassipes* may be necessary when it is incorporated into Nile tilapia diets at levels up to 20%.

Differences of $ADC_{protein}$ among plants could be attributed to the variances owing to the species. The population density, the ecological conditions, and the growth status of the plants at harvesting are all factors that affect their chemical composition. In fact, it must also be considered that plants were taken from natural water bodies instead of cultures.

Differences of ADC_{energy} and therefore digestible energy between treatments were found. All the energy values of the tested ingredients were relatively low and aquatic macrophytes can be described as a feed source poor in lipids and soluble carbohydrates, which do not possess desirable features to be used as an energy source. Interestingly, the ADC_{energy} obtained for both fermented (between 47.2% and 62.6%) and sundried macrophytes (between 37.8% and 51.1%) did not differ largely from those reported in the literature for common plant ingredients used in diets for *Piaractus brachypomus*. Gutiérrez-Espinosa & Vásquez-Torres (2008) reported similar ADC_{energy} for toasted whole soybean (59.1%) and soybean cake (59.9%). Closely related ADC_{energy} were reported by Vásquez-Torres *et al.* (2007) for palmiste (52.6 %), wheat bran (49.6 %) and sunflower cake (46.9%). Likewise, in diets for *P. mesopotamicus*, Abimorad & Carneiro (2004) also reported comparable ADC_{energy} for alcohol yeast (45.8%) and cottonseed meal (59.6%).

The low ADC_{energy} can be also attributed to the relatively high content of indigestible fibre in the tested aquatic macrophytes, since fibre might reduce the nutrient and energy availability of ingredients (Robinson & Li 2006). The most obvious difference between diets containing fish meal and alternative plant ingredients is the lower available digestible energy content.
Digestibility of non-conventional plant material is highly variable depending not only on the processing methods and the dietary inclusion level but on the tested fish species. Studies involving *Piaractus* regularly relate the high digestibility of several ingredients to morphological and histological advantages of the digestive system of this genus (Abimorad *et al.* 2007). *Piaractus brachypomus* is a typical omnivorous fish, although some aspects of its digestive tract morphology present similarities with carnivorous species as the trout, the Bacalao and the striped Bass. One of those characteristics is the presence of a clearly defined stomach and pyloric caeca (Muñoz *et al.* 2006). Further, Pacu possesses pharyngeal teeth (Muñoz *et al.* 2006), which participate in the crushing of diverse materials before reaching the stomach (Vásquez 2001). Also, as corresponded to omnivorous species, its intestine length is long compared to the standard length of fish (Muñoz *et al.* 2006).

The presence of the stomach is an important feature from a perspective of increased ability of fish to digest complex proteins and therefore to adapt to variable diets (Grabner & Hofer

1989), since the gastric glands present in the stomach secret pepsin and hydrochloric acid with the purpose to contribute to the enzymatic degradation of foods in the stomach (Kaye *et al.* 1995; Heath & Young 2000). Thus, fish species of the genus *Piaractus* are desired for using unconventional plant ingredients as nutrient sources in the rural aquaculture.

In conclusion, this study showed that $ADC_{drymatter}$, $ADC_{protein}$ and $ADC_{energy}$of fermented aquatic macrophytes were significantly higher than those of sundried aquatic macrophytes. Thus, fermented aquatic macrophytes, particularly *Spirodela polyrhiza, Lemna minor*, and *Azolla filiculoides* can be recommended as dietary ingredients into diets for the Cachama blanca (*Piaractus brachypomus*).

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CHAPTER 3.

Inclusion of fermented aquatic plants as feed resource for Cachama blanca, *Piaractus brachypomus*, fed low-fish meal diets

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Abstract

The production of the Amazonian fish Cachama blanca has been rising continuously and its cultivation has become heavily dependent on the provision of supplementary feed at low cost. Information on the suitability of locally available, cheap feed sources for this fish is required. The locally available duckweeds (*Lemna minor* and *Spirodela polyrhiza*) and water fern (*Azolla filiculoides*) were tested in Cachama blanca evaluating growth performance, feed conversion ratio, and digestibility. A total of five diets, four test diets (35% crude protein) supplemented with fermented duckweeds (DW) and water fern (WF) at 15% and 25% inclusion level and a control diet without aquatic plants, were compared. Ingredients were processed in a micro extruder. Diets were based on a low-fish meal diet. A total of 390 fish (1.6 ± 0.02 g) were randomly selected and stocked into fifteen 250 L plastic tanks providing three replicates per diet. Tanks were arranged in a recirculating system comprising a biofilter and aeration with a turn-over of and a daily water exchange. Fish were fed to apparent satiation twice a day for 60 days. Fish from each tank were weighed collectively every 2 weeks to monitor growth and after 8 weeks final biomass of each tank was recorded. Data from each treatment were subjected to one-way analysis of variance (ANOVA) of the triplicate groups (n=3). Fish fed on DW15 and WF15 revealed significantly higher ($P<0.05$) SGR and weight gain than fish fed the other diets. Feed intake did not vary among diets ($P>0.05$). FCR and PER were also better ($P<0.05$) for fish fed on DW15 and WF15 than for fish fed on DW25 and WF25 but not significant compared to the control diet. Apparent digestibility coefficients decreased significantly ($P<0.05$) in DW25 and WF25 diets. Fish feed supplementation with the fermented aquatic macrophytes at 15% inclusion level improved the growth performance of Cachamablanca (*P. brachypomus*) fed on low-fishmeal-diets.

Key Words: *digestibility, duckweeds, fermentation, growth performance, water fern.*

Resumen

La producción del pez amazónico Cachama blanca ha ido en continuo aumento y su cultivo se ha vuelto dependiente en gran medida de la disposición de alimento suplementario a bajo costo. Por eso se requiere información sobre la idoneidad de las fuentes de alimentación disponibles a nivel local que sean más económicas para este pez. Las lentejas de agua (*Lemna minor* y *Spirodela polyrhiza*) y el helecho de agua (*Azolla filiculoides*), disponibles localmente, fueron probadas en dietas para Cachama blanca evaluando el desempeño del crecimiento, conversión alimenticia y digestibilidad. Un total de cinco dietas: cuatro dietas de experimentación con un contenido de proteína cruda de 35%, suplementadas al 15% y 25% de inclusión con lentejas de agua (DW) y Azolla (WF) fermentadas, y una dieta control sin plantas acuáticas, fueron comparadas. Todos los ingredientes fueron procesados en una extrusora de micro-tornillo. Todas las dietas fueron basadas en un bajo contenido de harina de pescado. Un total de 390 peces (1,6 ± 0,02 g) fueron seleccionados al azar y distribuidos en quince tanques de plástico de 250 L con tres réplicas por dieta. Los tanques fueron dispuestos en un sistema de recirculación con un biofiltro y aireación constante y un recambio diario de agua. Los peces fueron alimentados hasta aparente saciedad dos veces al día durante 60 días. Los peces de cada tanque fueron pesados colectivamente cada 2 semanas para monitorear el crecimiento y después de 8 semanas se registró la biomasa final en cada tanque. Los datos de cada tratamiento fueron sometieron a un análisis de varianza (ANOVA) de los grupos por triplicado (n = 3). Los peces alimentados con las dietas DW15 y WF15 revelaron un crecimiento (SGR) y ganancia de peso significativamente mayor ($p < 0,05$) al de los peces alimentados con las otras dietas. El consumo de alimento no varió entre las dietas ($P > 0,05$). El FCR y PER fueron mejores ($P < 0,05$) para los peces alimentados con DW15 y WF15 que para los peces alimentados con DW25 y WF25 pero no hubo diferencias significativa en comparación con la dieta control. Los coeficientes de digestibilidad aparente disminuyeron significativamente ($P < 0.05$) en las dietas DW25 y WF25. El suplemento del alimento con las plantas acuáticas fermentadas al nivel de inclusión de 15% mejoró el desempeño del crecimiento de la Cachama blanca (*P. brachypomus*) alimentada con dietas de bajo contenido de harina de pescado.

Palabras claves: *digestibilidad, lenteja de agua, fermentación, crecimiento, Azolla*

Resumo

A produção do peixe amazônico Pirapitinga tem crescido continuamente e seu cultivo tornou-se dependente em grande medida da provisão de ração suplementar a baixo custo. Por este motivo se faz relevante a informação acerca de fontes de alimentação locais que sejam mais econômicas para a cultura deste peixe. As lentilhas de água (*Lemna minor* e *Spirodela polyrhiza*) e o feto de água (*Azolla filiculoides*), disponíveis localmente, foram testadas para a Pirapitinga avaliando o desempenho do crescimento, conversão alimentícia e digestibilidade. Um total de cinco dietas: quatro dietas de experimentação com um conteúdo de proteína crua de 35%, suplementadas a 15% e 25% de inclusão com lentilhas de água (DW) feto de água (WF) fermentados, e uma dieta de controle sem plantas aquáticas foram comparadas. Todos os igredientes foram processados numa micro extrusora. Todas as dietas foram baseadas em um conteúdo baixo de farinha de pescado. Um total de 390 peixes (1.6 ± 0.02 g) foram selecionados aleatoriamente e estocados em quinze tanques de plástico de 250 L com três réplicas por dieta. Os tanques foram foram dispostos em um sistema de re-circulação com um bio-filtro e aeração constante, e renovação diária de água. Os peixes foram alimentados até a saciedade aparente duas vezes ao dia durante 60 dias. Os peixes de cada tanque foram pesados coletivamente cada 2 semanas para monitorar o crescimento e depois de 8 semanas foi registrada a biomassa final de cada tanque. Os dados de cada tratamento foram submetidos a uma análise de variância (ANOVA) dos grupos triplicados ($n = 3$). Os peixes alimentados com as dietas DW15 e WF15 revelaram um crescimento (SGR) e ganho de peso significativamente maiores ($P<0.05$) ao dos peixes alimentados com as outras dietas. O consumo de alimento não variou entre as dietas ($P>0.05$). O FCR e PER foram melhores ($P<0.05$) para os peixes alimentados com DW15 e WF15 que para aqueles alimentados com DW25 and WF25 mas não houve diferença significativa em comparação com a dieta controle. Os coeficientes de digestibilidade aparente diminuíram significativamente ($P <0.05$) nas dietas DW25 e WF25. O suplemento do alimento com as plantas aquáticas fermentadas ao nível de inclusão de 15% melhorou o crescimento de crescimento da Pirapitinga (*P. brachypomus*) alimentada com dietas de baixo conteúdo de farinha de pescado.

Palavras-chave: *digestibilidade, lentilhas de água, fermentação, crescimento, Azolla*

Introduction

The tropical fish Cachama blanca (*Piaractus brachypomus*) plays an important role in the regional economy of the Amazon and Orinoco river basins from Colombia. Characterised by a delicate taste and a broad market acceptance it acquires good prices on the local market. As its natural populations do not support market demands, its aquaculture production increased substantially over the last years (Lochmann et al, 2009). Therefore an optimisation of culture techniques becomes priority in particular an efficient feeding, which represents a major expenditure in aquaculture operations in general.

In Colombia, which occupies the fifth place among the major aquaculture producing countries in Latin America with an aquaculture production of 60.072 tonnes/year (Florez-Nava, 2007), the production of Cachama has been rising continuously. At the same time, its cultivation has become heavily dependent on the provision of supplementary feed at low cost. As the majority of research has focused on plant ingredients commonly used in fish feed such as soybean and wheat derivates, information on the suitability of locally available, cheap feed sources for this fish is required.

Aquatic macrophytes are often considered as weeds in many aquatic environments as they grow very fast and cover the surface of ponds. In order to control their high productivity they are frequently harvested and used as livestock feed (Brabben 1993; Rodriguez and Preston 1996; Franklin et al., 2008). The suitability of aquatic macrophytes as feed sources in fish diets is highly dependent on the characteristics of the available plant species, their growth conditions and the species-specific requirements of fish. Aquatic macrophytes have not been yet assessed as dietary ingredients in diets for *Piaractus brachypomus*.

As many plant based ingredients, aquatic macrophytes are low digestible and are thus frequently further processed to increase their nutritional value. Processing of this plant material by lactic-acid fermentation has shown to reduce significantly the content of antinutritional substances and fibre present in the raw aquatic macrophytes (Cruz et al., 2011). As a result digestibility of the fermented aquatic plants is higher than that of the sundried aquatic plants for the Cachama blanca (Cruz et al., 2011). The aquatic macrophytes used in this study were fermented before inclusion in the diets.
The objective of this study was to investigate the effects of the inclusion of fermented aquatic macrophytes in low-fish meal diets (3% fishmeal) on the growth performance, feed utilisation and digestibility of Cachama blanca (*Piaractus brachypomus*) juveniles.

Material and Methods

Experimental diets

Five diets containing approximately 350 g kg^{-1} crude protein (CP) and 17 kJ/g gross energy (GE) were prepared. The control diet was formulated mainly with plant products (soy cake, corn gluten, rice bran and wheat bran), see Table 3.1.The maximum supplementation level (25%) of the fermented aquatic macrophytes in the experimental diets was calculated according to the apparent digestibility coefficients (ADC's) of the main protein ingredients, as determined by Vásquez-Torres (2007), and the ADC's of the fermented duckweeds (*Lemna* *minor*, *Spirodelapolyrhiza*) and water fern (*Azollafiliculoides),* as determined by Cruz et al. (2011), for Cachama blanca.

Table 3.1. Protein, lipid and energy content of the ingredients used for feed formulation based on a dry matter basis (g kg^{-1}).

Ingredients	Protein	Lipids	Carbohydrates	Energy (kJ g^{-1})
Fish meal[1]	717.3	90.0	20.00	21.3
Soy cake[1]	456.6	17.0	350.0	19.3
Corn gluten[1]	576.6	21.0	250.0	23.2
Rice bran[1]	123.7	15.0	550.0	8.96
Wheat bran[1]	146.9	36.0	615.0	19.5
Fermented duckweeds[2]	241.0	31.0	439.6	13.1
Fermented fern water[2]	264.0	31.0	442.6	11.5

[1] Obtained from ITALCOL Alimentos Concentrados © (Villavicencio, Colombia)

[2] Duckweed (*Lemna minor* and *Spirodelapolyrhiza*) and water fern (*Azollafiliculoides*) were harvested as wild or uncultivated material from water bodies in Colombia and afterwards fermented.

Table 3.2 shows the composition and nutritional content of the experimental diets. All diets contained 3% fish meal to enhance palatability and 0.5% of chromic oxide as inert marker. Aquatic macrophytes were included as fermented duckweeds at 15% (DW15) and 25% (DW25), and as fermented water fern Azolla at 15% (DW15) and 25% (DW25). Fermentation was carried out as described by Cruz et al. (2011) through the supplementation with Lactic acid bacteria (LAB) inoculants and molasses. The dietary ingredients, after mixing homogeneously, were processed in a micro extruder (Microextruder Exteect, Brazil) at temperatures of 65°C and pelleted to 4 mm diameter. The diets were dried at ambient temperatures within 4 hours and subsequently frozen at -4°C until utilized. The diets were prepared in the *Instituto de Acuacultura de los Llanos (IALL)* at the Universidad de los Llanos, Villavicencio (Colombia). The nutrient content of the diets was analysed according to AOAC (2005).

Table 3.2. Formulation and proximate composition of experimental diets with macrophytes (DW – duckweed, WF – water fern) as alternative, cheap feedstuff at 15% and 25% and the control used in an 8 week feeding trial in Cachamablanca.

Ingredients	Control Diet	DW15	DW25	WF15	WF25
Fish meal	30.0	30.0	30.0	30.0	30.0
Soy cake	250.0	250.0	250.0	250.0	250.0
Corn gluten	200.0	200.0	200.0	200.0	200.0
Casein	5.0	0.0	0.0	0.0	0.0
Rice bran	50.0	50.0	50.0	50.0	50.0
Wheat bran	365.0	160.0	0.0	150.0	0.0
Fermented duckweeds	0.0	150.0	250.0	0.0	0.0
Fermented water fern	0.0	0.0	0.0	150.0	250.0
Alfa-cellulose	0.0	60.0	120.0	70.0	120.0
Carboxymethyl cellulose	25.0	25.0	25.0	25.0	25.0
Fish oil	20.0	20.0	20.0	20.0	20.0
Sunflower oil	20.0	20.0	20.0	20.0	20.0
Vitamin premix[1]	10.3	10.3	10.3	10.3	10.3
Mineral premix[2]	10.3	10.3	10.3	10.3	10.3
Ascorbic acid (Stay C-35)[3]	5.0	5.0	5.0	5.0	5.0
Cr_2O_3	5.0	5.0	5.0	5.0	5.0
Proximate Composition					
Dry Matter	920.8	933.6	938.6	916.3	933.5
Ash	67.2	72.5	76.1	81.9	95.6
Crude Protein	357.8	354.1	344.0	353.5	344.5
Ether Extract	56.1	53.3	60.7	51.3	74.7
Crude Fiber	68.2	100.1	133.7	95.6	137.2
NFE[4]	450.7	420.0	385.5	417.7	348.0
GrossEnergy (kJg-1)	18.4	17.7	17.6	17.2	17.1

[1]Rovimix vitamin: ®Lab. Roche S.A. 0.5 (Vit A 8.0*106 UI, Vit D3, 1.8*106 UI, Vit E 66.66g, Vit B1 6.66g, Vit B2 13.33g, Vit B6 6.66g, Calcium pantothenic 33.33g, Biotin 533.3mg, Folicacid 2.66g, Ascorbicacid 400.0g, Nicotinicacid 100.0g, Vit B12 20.0mg, Vit K3 6.66g, cspvehicle 1.0Kg.

[2]Micro-minerals premix: ®Lab. Roche S.A. 1.0 (Composition per 100g the product: Mg 1.0, Zn 16.0, Fe 4.0, Cu 1.0, I 0.5, Se 0.05, Co 0.01).

[3]Vitamin C, StayC-35

[4]Nitrogen-free Extract (NFE) = 100-(Ash+ Protein+ Fibre+ Fat)

Culture system and experimental design

A total of 390 Cachama blanca fingerlings were obtained from a regional producer in Villavicencio (Colombia) and transported to the Instituto de Investigaciones Tropicales at the Universidad del Magdalena (Colombia). Fish were fed a commercial diet for 4 weeks prior to stocking in order to adapt them to the experimental conditions. Following fish were randomly selected and stocked into fifteen 250 L plastic tanks providing three replicates per diet. Individual initial weight of fish was 1.6±0.02 g (mean±SD). Tanks were arranged in a recirculating system comprising a biofilter and aeration with a turn-over of and a daily water exchange. Water parameters were monitored weekly (26.9±0.93 °C water temperature and 160.2±10.4 mg/L $CaCO_3$ hardness).

Sampling

Fish were fed to apparent satiation twice at day (0900 and 1700 h) for 60 days. Fish from each tank were weighed collectively every 2 weeks to monitor growth and after 8 weeks final biomass of each tank was recorded. At the end of the experimental period, three fish from each replicate were sampled as bulk sample (n=3 per treatment) for carcass analysis and stored at –20°C. For each treatment (n=3), survival, growth parameters, specific growth rate (SGR), weight gain (WG), feed conversion ratio (FCR) and protein efficiency ratio (PER) were determined according to the formulas in Table 3.3.

Apparent nutrient digestibility

After the 8 weeks experimental period, a 3 weeks study on the apparent digestibility was conducted providing the diets used in the feeding trial, but feeding only once daily at 0900 h. Faeces were collected by siphoning from the bottom of the tanks at intervals of 1 h over a period of 8 h post-feeding or after feeding. The ADC's of dry matter, ash and protein of the test diets were calculated using chromic oxide (Cr_2O_3) as inert marker following the formula described by Nose (1960):

$$ADC_{diet} = 100 - \left\lfloor 100 \times (\%Cr_2O_{3diet} / \%Cr_2O_{3faeces}) x(\%Nut_{faeces} / \%Nut_{diet}) \right\rfloor$$

Where:

ADC_{diet} = Apparent digestibility coefficient of the nutrients or energy in diets

$\%Cr_2O_{3diet}$ = % of chromium content in diets

$\%Cr_2O_{3faeces}$ = % of chromium content in faeces

$\%Nut_{diet}$ = % of nutrient or energy in diets

$\%Nut_{faeces}$ = % of nutrient or energy in faeces

Chemical analyses

Analysis of dry matter, ash, lipids, crude fibre and crude protein for diets, faeces and muscle were made according to the AOAC (2005) procedures. Gross energy (GE) was determined by using an adiabatic bomb calorimeter (PARR 121 EA, USA). Spectro photometrically determination of chromic oxide in diets and faeces was performed following the method of Furukawa and Tsukahara (1966).

Statistical analyses

Data from each treatment were subjected to one-way analysis of variance (ANOVA) and are presented as mean ± standard deviation (SD) of triplicate groups (n=3). Analysis was carried out with SPSS 17.0 (Version 19) software package and data were analysed for normal distribution by Kolmogorov-Smirnov and homogeneity of variances by Levene Test (passed if $p<0.05$). For multiple comparasion, either parametric Tukey's multiple range or nun-parametric Dunnett's T3 tests were carried out.

Results

Water quality parameters were optimal for Cachama blanca, resulting in a high survival between 94% and 97%. The effect of the diets on growth and feed utilization is shown in Table 3.3. Acceptance of all diets was very good as active, complete consumption were observed and was comparable between treatments.

Table 3.3. Growth, feed utilization and survival in juvenile Cachama blanca (*Piaractus brachypomus*) fed the experimental diets using macrophytes (DW – duckweed, WF – water fern). Values with the same superscript are not significantly different (p>0.05).

Variables	Diets				
	Control	DW15	DW25	WF15	WF25
Wi (g)	1.57 ± 0.04	1.61 ± 0.03	1.58 ± 0.03	1.61 ± 0.06	1.61 ± 0.11
Wf (g)	11.27 ± 0.65 ^{b}	13.78 ± 0.34 ^{a}	10.90 ± 0.74 ^{b}	13.90 ± 0.46 ^{a}	10.88 ± 1.04 ^{b}
SGR[1] (%/d)	3.29 ± 0.06 ^{b}	3.57 ± 0.03 ^{a}	3.22 ± 0.13 ^{b}	3.59 ± 0.02 ^{a}	3.18 ± 0.13 ^{b}
WG[2] (%)	9.70 ± 0.62 ^{b}	12.17 ± 0.32 ^{a}	9.32 ± 0.75 ^{b}	12.29 ± 0.41 ^{a}	9.26 ± 0.97 ^{b}
FCR[3]	1.19 ± 0.08 ^{ab}	1.06 ± 0.03 ^{a}	1.37 ± 0.16 ^{b}	1.05 ± 0.09 ^{a}	1.34 ± 0.06 ^{b}
PER[4]	2.35 ± 0.17 ^{ab}	2.66 ± 0.07 ^{a}	2.08 ± 0.23 ^{b}	2.78 ± 0.24 ^{a}	2.18 ± 0.10 ^{b}
FC[5] (gfish^{-1})	11.53 ± 0.74	12.91 ± 0.22	12.69 ± 0.63	12.93 ± 1.56	12.34 ± 0.76
SR[6] (%)	94.87 ± 5.88	97.44 ± 2.22	94.87 ± 4.44	94.87 ± 5.88	93.59 ± 4.44

[1]Specific growth rate (SGR) = [lnWf (mean final weight) − lnWi (mean initial weight)/56 days] × 100.

[2]Percent weight gain (WG) = 100(Final weight-Initial weight)/ Initial weight.

[3]Feed conversion ratio (FCR) = total feed intake in dry basis (g) / wet weight gain (g).

[4]Protein efficiency ratio (PER) = total weight gain (g)/protein intake (g).

[5]Feed consumption (FC) during the experimental period (56 days)

[6]Survival Rate (SR)

Wi: initial weight, Wf: final weight

Results indicate that feed supplementation by the fermented aquatic macrophytes at 15% of inclusion level enhances growth of Cachama blanca fed low fish meal diets. Fish fed DW15 and DW15 diets revealed the highest growth performance, including SGR, final weight and weight gain (P<0.05) compared to the control, DW25 and DW25 diets respectively (Table 3.3). Figure 1 describes growth characteristics in two weeks intervals over the 8 week trial, revealing significant high growth performance for DW15 and WF15, from week 4 on.

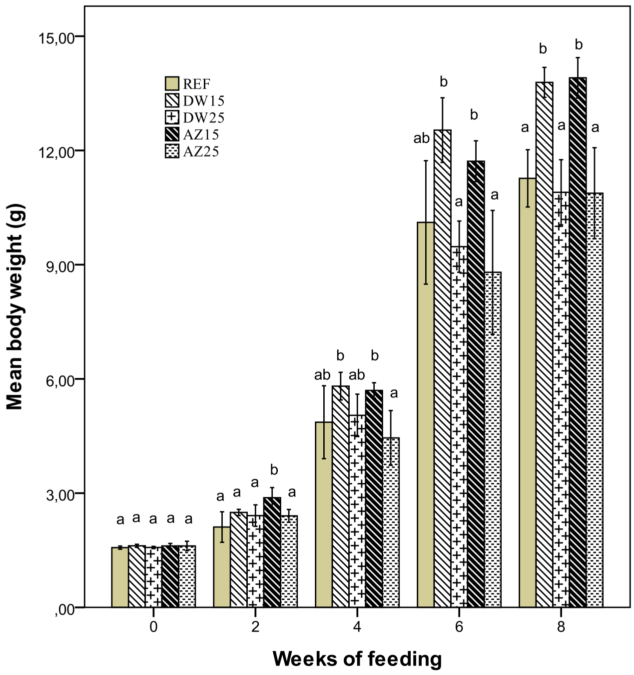

Figure 3. Body weight in juvenile Cachama blanca (*Piaractus brachypomus*) fed the experimental diets using macrophytes (DW – duckweed, WF – water fern). Values with the same superscript are not significantly different (p>0.05).

The feed utilization, as assessed in FCR and PER, showed no significant differences among DW15, WF15 and the control diet (n=3, P>0.05), but it was significantly higher (P<0.05) than for DW25 and WF25 diets. Congruently, apparent digestibility coefficients (ADC) of dry matter, ash and protein were higher (P>0.05) for DW15, WF15 and the control diet, compared to DW25 and WF25 diets (Table 3.4). And ADC of lipids was significantly higher in the DW25 and DW25 diets compared to the DW15 and DW15 diets.

Table 3.4. Apparent digestibility coefficients (ADC, %) in juvenile Cachama blanca (*Piaractus brachypomus*) fed the experimental diets using macrophytes (DW – duckweed, WF – water fern).Values with the same superscript are not significantly different (p>0.05).

	Diets				
Variables	Control	DW15	DW25	WF15	WF25
ADC_dry matter	93.79 ± 0.39^{a}	92.11 ± 1.76^{a}	68.89 ± 7.84^{b}	93.70 ± 2.28^{a}	75.00 ± 5.40^{b}
ADC_ash	91.13 ± 0.61^{a}	89.09 ± 2.35^{a}	45.88 ± 8.54^{b}	90.39 ± 3.38^{a}	56.12 ± 5.96^{b}
ADC_protein	98.34 ± 0.08^{a}	98.64 ± 0.28^{a}	87.56 ± 3.06^{b}	98.33 ± 0.48^{a}	88.30 ± 2.78^{b}
ADC_lipids	88.37 ± 0.38^{b}	88.45 ± 0.09^{b}	92.81 ± 0.11^{a}	87.49 ± 0.10^{c}	88.68 ± 0.42^{b}

Discussion

To make the diets isonitrogenous it was necessary to balance the supplementation of 15 or 25% of water plants by alpha cellulose in the experimental diets. As a result the gross energy decreased in the experimental diets from 4 to 7% and the fibre content increased from 40 to 100%. Despite the lower energy concentration of the experimental diets the protein was adequately absorbed and not used as energy source, which is indicated by the significant higher growth in DW15 and WF15 compared to the control. As the content of soluble carbohydrates as energy source was diminished in the experimental diets, the energy need was compensated by an increase in feed intake by aproximately 11% in DW15 and WF15. At the 25% diets the nutrient imbalance cannot be compensated by a higher feed intake.

The SGR reported in this study from 3.2 to 3.6 for diets at 25% and 15% inclusion level, respectively, were comparable to the results reported by Palacios et al. (2006) for Cachama blanca fed a semi-purified diet supplemented at 15% with the Peruvian plants aguaje fruit (3.6), and maca meal (4.1) and higher than those reported for fish fed camu-camu fruit (1.5), which presented a very low SGR. In other study Gaitán-Ibarra (2008) reported also lower SGR for Cachama blanca fed diets supplemented with probiotics and yeast. These results support that fermented aquatic plant supplementation at 15% level into a practical diet increases growth rates and feed efficiency in Cachama blanca.

Fish fed diets containing non-conventional plant material result commonly on poor growth. This is frequently attributed to the presence of antinutrients, low palatability of plant material and the subsequent reduced feed intake. As fermentation process reduced effectively the content of common antinutrients present in the aquatic macrophytes, as we could analyze in an experiment (Cruz et al, 2011), and feed intakes in this study have not differed, the decreased digestibility of dry matter and protein could not be negatively affected by antinutrients but by the higher ash content of each aquatic macrophytes and the 37% increased fibre content in the 25% diets. Similar results have been congruently observed in previous studies on aquatic macrophytes used as feed ingredient for other tropical fish (Bairagi et al, 2002; El-Sayed, 2003, Yılmaz et al, 2004).

Despite of the available protein content of the experimental diets fulfil the requirements of Cachama blanca and to the higher fat content and fat digestibility in the 25% supplementation groups, the reduced availability of nutrients in these diets could not compensate the deficit of energy.

The FCR values from 1.05 to 1.37 in the present study were better than those derived from other alternatives plant sources such as wheat bran, uncooked and cooked yucca, pijuayo, and plantain, which were reported by Lochmann et al. (2009). Gaitán-Ibarra (2008) observed similar FCR values from 1.31 to 2.50 for probiotics and yeast supplemented diets. Better FCR values from 0.64 to 0.68 were only reported by Palacios et al. (2006) for Cachama blanca fed semipurified diets supplemented with the Peruvian plants maca meal and aguaje fruit, respectively.

The potential variability in the feed utilization parameters between aquatic macrophytes and the Peruvian plants, maca meal and aguaje fruit, may be related to the use of semipurified diets versus the practical ingredient diets in the present study. In their study on digestibility of diet ingredients for Cachama blanca Fernandes et al. (2004) suggested that interactions between diet components are different in semipurified and practical diets and therefore the availability of the nutrients and energy can vary.

Lipids digestibility was higher for diets containing aquatic macrophytes at 25% level. Consequently, it might be due to a lower content of total carbohydrates in DW25 and WF25 diets, then Cachama blanca utilized lipids as energy source for growth. It is consistent with a study in a close relative species Pacu (*P. mesopotamicus*), which is able to utilize lipids as well as carbohydrates to spare protein for somatic growth (Abimorad and Carneiro, 2007). In addition, we also assumed that a dependence between lipids and carbohydrates levels occurred, but further research is needed for confirmation. In general, digestibility coefficients of control and 15% diets were comparable to those reported for common ingredients for pacu (Fernandes et al., 2004).

In conclusion, the utilisation of fermented macrophytes as a cheap, local ingredient for fish feed formulation was feasible up to 15% of inclusion level; thereby their use could support small-scale farming of Cachama blanca in rural zones.

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CHAPTER 4.

Effect of fermented aquatic macrophytes supplementation on growth performance, feed efficiency and digestibility of Nile Tilapia (*Oreochromis niloticus*) juveniles fed low fishmeal diets

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Abstract

An 8-week feeding experiment was conducted to evaluate the growth performance, feed efficiency, digestibility, carcass composition and, liver and intestine histology of juvenile Nile Tilapia (3.18 ± 0.02 g) fed on a control diet and four experimental diets (35% CP). The experiment aimed to assess the utilization of local macrophytes; namely fermented duckweeds at 15% (DW15) and 25% (DW25) as well as fermented water fern at 15% (WF15) and 25% (WF25). The growth performance comprising of weight gain (WG), specific growth ratio (SGR) and protein efficiency ratio (PER) was assessed in triplicates and no significant differences between diets were observed. Feed conversion ratio (FCR) and ash content of the carcass were similar in all the treatments except for WF25 where significantly higher FCR and ash content were recorded compared to the control diet. However, lipid and protein content of the carcass was comparable between diets. Results showed that the inclusion of fermented duckweeds up to 25% and fermented water fern up to 15% in diets for Nile Tilapia was feasible without any negative effect compared to the control group.

Key Words: *duckweed, growth performance, histology, Nile Tilapia, plant ingredients, water fern.*

Introduction

In contrast to Asia, where aquaculture industry is primarily dependent on farm-made feeds, Latin America's industry is mainly dependent on manufactured feeds. Most operations of aquaculture industry in Latin America consist of semi intensive and intensive production systems involving the subsequent use of commercial aquafeeds based on fish meal (Flores-Nava, 2007). During the last years, domestic fish consumption and fish production in the region increased strongly (Flores-Nava, 2007), consequently increasing the demand for aquafeeds. Thus, industrially manufactured feed has become more readily available in most Latin American countries, where it is produced by large manufactures. In contrast, small-scale producers in rural areas do not have the capital for these costly commercial feeds and farm-made feeds are only occasionally produced in localized regions where locally grown agricultural by-products are utilized to replace or complement cost commercial diets (Hasan et al, 2007). A basic knowledge in the use of alternative ingredients, especially in plant based sources, could therefore significantly contribute to the production of low cost, farm-made feeds for local fish production.

Imbalanced essential amino acid profile, the presence of antinutritional substances, low digestibility and poor palatability are the most common problems encountered when using plant sources as fish feed ingredients (Lovell, 1989; Tacon, 1994; Francis et al, 2001). For a successful inclusion of plant based ingredients into diets, it is necessary to evaluate growth performance and feed conversion, and carefully monitor the potential adverse effects on the fish physiology. Aquatic macrophytes are known to be among the potential candidates for local production of aquafeeds (El-Sayed, 2003; Bairagi et al, 2002; El-Sayed, 1999; Leng et al, 1995). Moreover, they are widely used as feed for cultured omni-herbivorous fish in many Asian countries where aquaculture is primarily rural (Hasan and Chakrabarti, 2009) and finances do not allow for expensive feed sources. Usually, they are processed either as sun dried material or after ensiling in order to improve their digestibility by fish (El-Sayed, 2003; Bairagi et al, 2002).
Due to its nutritional requirements, Tilapia can be grown on plant-based diets including algae, aquatic plants and a variety of feeds from diverse origins (Watanabe, 2002) and thus it is relatively inexpensive. This makes it particularly beneficial in meeting the needs of small-scale farms in rural Colombia. A number of studies have been carried out on the use of different macrophytes in Tilapia feed (Mandal et al, 2010; Abdelhamid et al, 2010; Abdel-Tawwab, 2008; Abdelhamid et al, 2006; El-Sayed, 2003; Fasakin et al, 1999).

Tilapia is consequently an ideal species for aquaculture and it is in high demand in the majority of the international and domestic markets. In the many countries of Latin America where Tilapia has been introduced, it plays an important role as high quality food source, especially in rural communities. In Colombia's national aquaculture production, Tilapias occupy the first place (principally red Tilapia), followed by Cachama production (*Piaractus brachypomus*) (Espinal et al., 2005, FAO, 2012). Unfortunately, its production in rural regions is limited due to the high costs of fish feeds.

In an effort to minimize the use of expensive ingredients and reduce the production costs of Tilapia in small-scale aquaculture systems, the present study aims to evaluate the utilization of fermented locally available aquatic macrophytes in a low fish meal content (3 % DM) diet.

Material and Methods

Experimental diets

A total of five isonitrogenous (35 % crude protein) and isoenergetic (17 kJ g^{-1} gross energy) diets were formulated. Soy cake and corn gluten were used as the main plant protein sources for the diets. Due to their comparable nutrient composition wheat bran was replaced by fermented duckweeds (*Lemna minor* and *Spirodela polyrhiza*) at 15 % (DW15) and 25 % (DW25) and by fermented water fern (*Azolla filiculoides*) at 15% (WF15) and 25 % (WF25) inclusion levels. In each diet 3 % fishmeal was included to assure palatability of the experimental diets. To maintain the level of protein in the diet as well as the low content of fishmeal, wheat bran and alphacellulose were removed as fermented duckweed or fern were added.

Aquatic macrophytes were fermented using a commercial silage LAB inoculants based on the bacterial strain *Lactobacillus plantarum* DSM 8862 and DSM 8866 (BIO-SIL®, Dr. Pieper Technologie-und Produktentwicklung GmbH, Germany). For fermentation, freshly harvested plants were mixed with a part of dried aquatic macrophytes of the same sample until about 350 to 450 g kg-1 dry matter content was obtained. Molasses at 15 % was added and the mixture was vacuum packed into plastic bags according to the method described by Johnson et al. (2005). After four weeks plastic bags were opened and samples were dried in an oven at 45 °C for 48 h. Afterwards proximal composition of the fermented plant material was determined. Fermented macrophytes were then used to prepare the experimental diets. All ingredients of the diets were well grounded, mixed and finally pelleted to pieces of 4 mm in diameter. Chromic oxide at 0.5 % was added as an inert marker.

The proximate composition of the tested diets is shown in Table 4.1. The most prominent difference between diets was the increased ash and crude fibre content in the DW and WF diets compared to the control diet. Between experimental diets WF showed stronger increase in ash content (by 5% and 17% respectively) than DW at corresponding inclusion levels. In contrast, the fibre content depended only on the inclusion level of aquatic macrophytes and was found to be higher than in the control diet. Lipid content was relatively low but comparable in all diets.

Growth trial

The experiment was carried out at the aquaculture facilities of Humboldt University, Berlin. A total of 225 juveniles of Nile Tilapia (*Oreochromis niloticus*) with a initial body mass of 3.18 ± 0.02 g (mean ± SD) were randomly distributed into fifteen 200L flow-through glass aquaria at a density of 15 fish per aquarium, providing three replicates for each experimental diet. Water parameters were monitored weekly and were within optimum ranges for normal growth and health of Tilapia juveniles according to Stickney (1979): 25.3 ± 1.07 °C, pH 8.43 ± 0.17, < 0.3 mgl^{-1} NO_2, < 0.01 mgl^{-1} NH_4,, 8.79 ± 0.57 mgl^{-1} dissolved oxygen respectively.

Fish were fed a commercial diet for two weeks of acclimatisation. Afterwards, the experimental diets were randomly assigned to the aquaria. Fish were handfed to apparent satiation for 56 days, twice a day at 09:00 and 17:00 h. Fish were weighed in bulk every two weeks and additionally, the total length and weight were measured individually at the end of the trial. During the experiment a photoperiod of 12 h light: 12 h darkness was maintained. At the end of the experiment three fish from each aquarium were sampled as bulk samples (n = 3 per treatment) for carcass analysis and stored at –20 °C.
Additionally, six individuals from each dietary group were dissected and samples of the liver and the intestine were weighed and taken for histological analysis.

Apparent nutrient digestibility

During the last three weeks of the experimental period, faecal samples were collected twice from each aquarium between the feeding times at 1 h intervals. Samples were collected using a filtering net, rinsed in distilled water, then transferred to a filter paper, oven dried at 60 °C for 72 h and stored until laboratory analysis. Apparent digestibility coefficients (ADC) of dry matter, ash, protein and lipid were calculated using chromic oxide (Cr_2O_3) as inert marker following the formula described by Nose (1960):

$ADC_{diet} = 100 - \lfloor 100 \times (\%Cr_2O_{3\,diet} / \%Cr_2O_{3\,faeces}) x (\%Nut_{faeces} / \%Nut_{diet}) \rfloor$, where:

ADC_{diet} =Apparent digestibility coefficient of the nutrients or energy in diets

$\%Cr_2O_{3\,diet}$ = % of chromium content in diets

$\%Cr_2O_{3\,faeces}$ = % of chromium content in faeces

$\%Nut_{diet}$ = % of nutrient or energy in diets

$\%Nut_{faeces}$ = % of nutrient or energy in faeces

Chemical analyses

Proximate analysis of the diets, faeces and carcass was performed following AOAC (2005) procedures. Gross energy (GE) was determined by using an adiabatic bomb calorimeter (Parr 121 EA, USA). Chromic oxide in diets and faeces was determined spectrophotometrically by the method of Furukawa and Tsukahara (1966). All samples were analyzed in duplicates.

Histological analyses

For histological analysis, six fish per dietary group were dissected and the liver as well as the sac-like stomach was sampled. Macroscopically, no distinct regions of the stomach were sampled in order to standardize sampling with regard to controversial reports on distinct regions of the stomach in Nile Tilapia *O. niloticus* (Caceci et al. 1997; Osman & Caceci, 1991, Al-Hussaini & Kholy, 1953). Samples were fixed in 10 % phosphate-buffered formalin, gradually dehydrated, cleared, embedded in paraffin, cut to 5 µm slices using a microtome (Leica RM 2135) and stained with haematoxylin and eosin (HE). Analysis was carried out with a light microscope (Leica DM 2500) equipped with a digital camera (Leica DFC 420). For the histological analysis any alterations and abnormalities were recorded. During histological analysis, samples of liver and stomach from fish *O. niloticus* fed standard commercial feed (standard group) were compared to the fish fed control and test diets.

Data calculation and Statistical analyses

The criteria used to determinate growth performance, feed utilization and morphological measurements were:

Specific growth rate (SGR) = [ln Wf (mean final weight) − ln Wi (mean initial weight)/days (d)] × 100

Percent weight gain (WG) = 100(Final weight-Initial weight)/ Initial weight

Feed conversion ratio (FCR) = total feed intake in dry basis (g) / wet weight gain (g)

Protein efficiency ratio (PER) = total weight gain (g)/protein intake (g)

Hepatosomatic Index (HSI) = [Liver mass (g) / body mass (g)] × 100

Intestinal Somatic Index (ISI) = [Intestine mass (g) / body mass (g)] × 100

Data from each treatment were subjected to one-way analysis of variance (ANOVA) and are presented as mean ± standard deviation (SD) of triplicate groups (n=3). Data were analysed for normal distribution by Kolmogorov–Smirnov and equal variance by Levene Test (passed if $p < 0.05$) using SPSS 17.0 for *Windows*. For multiple comparison, parametric Tukey's multiple range test or non-parametric Dunnett`s T3 were used. Individuals were sampled from each replicate for histology analysis.

Results

Water quality parameters were optimal for Nile Tilapia and the percentage of survival rate ranged from 95.3 % to 100 % (Table 4.2). The parameters of growth, final weight (Wf), weight gain (WG) and specific growth rate (SGR) ranged from 15.5 to 16.6 g, 377-419 % and from 2.8 to 2.9 g day^{-1} and were not significant. DW15 and WF15 had a tendency to show higher values than control and the 25% supplementation group (Figure 4). In contrast, the feed efficiency parameters were significantly different displaying better values for control group and DW15. This is a result of higher feed consumption in DW25 and WF15 and WF25 (up to 140% in WF25).

Table 4.1: Composition and nutrient content of the experimental diets with macrophytes (DW – duckweed, WF – water fern) as alternative, cheap feedstuff at 15% and 25% and the control used in an 8 week feeding trial in Nile tilapia (*Oreochromis niloticus*) juvenile on a dry matter basis (g kg^{-1}).

Ingredients	Control	DW15	DW25	WF15	WF25
Fish meal	30.0	30.0	30.0	30.0	30.0
Soy cake	250.0	250.0	250.0	250.0	250.0
Corn gluten	200.0	200.0	200.0	200.0	200.0
Casein	5.0	0.0	0.0	0.0	0.0
Rice bran	50.0	50.0	50.0	50.0	50.0
Wheat bran	365.0	160.0	0.0	150.0	0.0
Fermented duckweeds	0.0	150.0	250.0	0.0	0.0
Fermented water fern	0.0	0.0	0.0	150.0	250.0
Alfa-cellulose	0.0	60.0	120.0	70.0	120.0
Carboxymethyl cellulose	25.0	25.0	25.0	25.0	25.0
Fish oil	20.0	20.0	20.0	20.0	20.0
Sunflower oil	20.0	20.0	20.0	20.0	20.0
Vitamin premix[1]	10.3	10.3	10.3	10.3	10.3
Mineral premix[2]	10.3	10.3	10.3	10.3	10.3
Ascorbic acid (Stay C-35)[3]	5.0	5.0	5.0	5.0	5.0
Cr_2O_3	5.0	5.0	5.0	5.0	5.0
Proximate Composition					
Dry Matter	920.8	933.6	938.6	916.3	933.5
Ash	67.2	72.5	76.1	81.9	95.6
Crude Protein	357.8	354.1	344.0	353.5	344.5
Ether Extract	56.1	53.3	60.7	51.3	74.7
Crude Fiber	68.2	100.1	133.7	95.6	137.2
NFE[4]	450.7	420.0	385.5	417.7	348.0
Gross Energy (kJg-1)	18.4	17.7	17.6	17.2	17.1

[1]Rovimix vitamin: ®Lab. Roche S.A. 0.5 (Vit A 8.0*106 UI, Vit D3, 1.8*106 UI, Vit E 66.66g, Vit B1 6.66g, Vit B2 13.33g, Vit B6 6.66g, Calcium pantothenic 33.33g, Biotin 533.3mg, Folic acid 2.66g, Ascorbic acid 400.0g, Nicotinic acid 100.0g, Vit B12 20.0mg, Vit K3 6.66g, csp vehicle 1.0Kg.

[2]Micro-minerals premix: ®Lab. Roche S.A. 1.0 (Composition per 100g the product: Mg 1.0, Zn 16.0, Fe 4.0, Cu 1.0, I 0.5, Se 0.05, Co 0.01).

[3]Vitamin C, StayC-35, [4]Nitrogen-free Extract (NFE) = 100-(Ash+ Protein+ Fibre+ Fat)

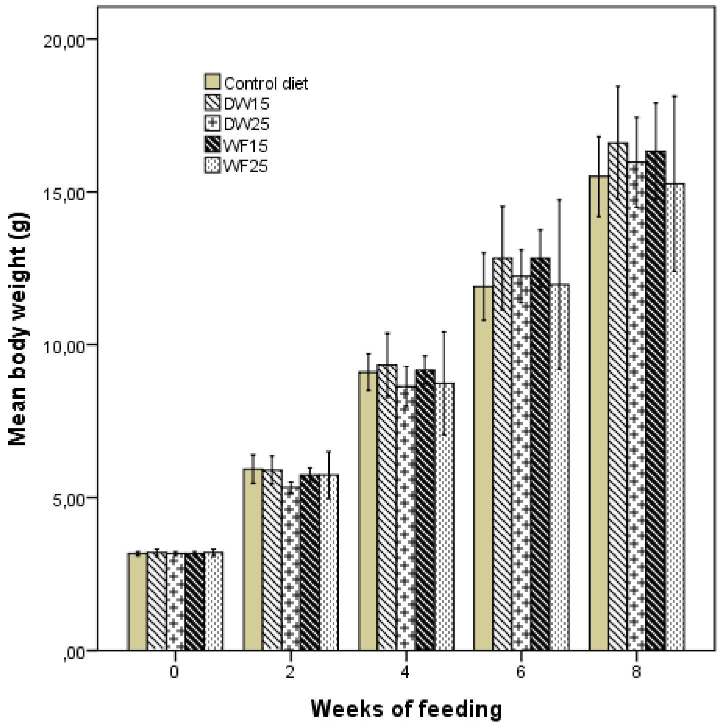

Figure 4: Mean body (mean ± SE, n=3) weight of Nile Tilapia fed experimental diets for 8 weeks. No significant differences between experimental groups were detected.

Table 4.2: Growth performance, feed efficiency and survival rate of juvenile Nile Tilapia (mean ± SD) of low fish meal diets (3 %) comprising a control and low cost diets with duckweed (DW) or water fern (WF) at 15 % or 25 % of the crude protein. Values with a different superscript are significantly different ($p < 0.05$, n= xy). Tukey Test, Dunnett`s T3 Test.

	Control	DW15	DW25	WF15	WF25
Wi (g)	3.2 ± 0.03	3.2 ± 0.06	3.2 ± 0.03	3.2 ± 0.03	3.2 ± 0.06
Wf (g)	15.5 ± 0.65	16.6 ± 0.93	16.0 ± 0.74	16.3 ± 0.79	15.3 ± 1.43
SGR[1] (% day^{-1})	2.8 ± 0.09	2.9 ± 0.10	2.9 ± 0.10	2.9 ± 0.07	2.8 ± 0.15
WG[2] (%)	388.5 ± 19.2	418.7 ± 30.2	405.3 ± 22.7	415.0 ± 20.9	377.2 ± 38.9
FCR[3]	1.8 ± 0.06 ^{a}	1.7 ± 0.07 ^{a}	2.1 ± 0.18 ^{ab}	2.1 ± 0.15 ^{ab}	2.6 ± 0.20 ^{b}
PER[4]	1.6 ± 0.06 ^{a}	1.6 ± 0.09 ^{a}	1.4 ± 0.12 ^{ab}	1.4 ± 0.09 ^{ab}	1.1 ± 0.09 ^{b}
FC[5] (g fish^{-1})	21.9 ± 1.3 ^{a}	22.9 ± 0.8 ^{ab}	26.2 ± 3.0 ^{ab}	27.3 ± 0.8 ^{b}	30.5 ± 1.7 ^{b}
SR (%) [6]	95.3 ± 2.3	100 ± 0.0	95.7 ± 4.3	100 ± 0.0	100 ± 0.0

[1]Specific growth rate (SGR) = [lnWf (mean final weight) − lnWi (mean initial weight)/56 days] × 100.
Wi: initial weight, Wf : final weight

[2]Percent weight gain (WG) = 100(Final weight-Initial weight)/ Initial weight.

[3]Feed conversion ratio (FCR) = total feed intake in dry basis (g) / wet weight gain (g).

[4]Protein efficiency ratio (PER) = total weight gain (g)/protein intake (g).

[5]Feed consumption (FC) during the experimental period (56 days).

[6]Survival Rate (SR)

Digestibility analysis (Table 4.3) revealed that dry matter, ash, lipid and protein digestibility was highest in the control at 88 %, 84 %, 98 %, 96 % followed by 15 % inclusions. All nutrients showed significantly lower digestibility in the 25 % groups compared to control group. In comparing the two aquatic plants, DW groups presented the tendency to have higher ADC values than WF groups at the same inclusion levels.

Table 4.3: Apparent digestibility coefficients (ADC, %) of the tested diets for Nile Tilapia (means ± SD) of low fish meal diets (3 %) comprising a control and low cost diets with duckweed (DW) or water fern (WF) at 15 % or 25 % of the crude protein. Values with a different superscript are significantly different ($p < 0.05$, n= xy). Tukey Test, Dunnett`s T3 Test.

	Control	DW15	DW25	WF15	WF25
ADC_dry matter	88.09 ± 4.48^{a}	79.68 ± 6.72^{a}	57.80 ± 5.53^{b}	79.22 ± 8.97^{a}	50.08 ± 5.13^{b}
ADC_ash	84.50 ± 5.89^{a}	73.74 ± 8.62^{a}	40.31 ± 7.98^{b}	70.31 ± 12.96^{a}	27.95 ± 7.61^{b}
ADC_protein	97.96 ± 0.81^{a}	95.77 ± 1.41^{a}	88.02 ± 1.69^{bc}	93.12 ± 2.94^{ab}	84.53 ± 2.07^{c}
ADC_lipids	96.53 ± 0.74^{a}	95.20 ± 0.36^{a}	88.63 ± 2.19^{b}	83.87 ± 0.31^{c}	80.62 ± 0.94^{d}

The proximate carcass composition and morphological measurements of Nile Tilapia are presented in Table 4.4. There were no differences in the carcass protein (39.3 - 40.5 %) and carcass lipid content (20.4 - 22.1 %) between all treatments. The carcass ash content in group WF25 (7.1 %) was significantly higher than in the control group (6.6 %). No significant differences between the dietary groups were recorded in the ISI ranging from 4.97 (WF15) to 5.51 (WF25). Whereas, low HIS was observed at high macrophyte incorporations, in WF25 and DW25, 1.80 and 1.88, respectively, but a significant difference to the control was not detected.

Table 4.4: Carcass composition (on a wet weight basis, g kg^{-1}) hepatosomatic (HIS) and intestinal somatic index (ISI) morphological measurements of Nile Tilapia fed on tested diets (mean ± SD). Values with a different superscript are significantly different ($p < 0.05$, n= xy). Tukey Test, Dunnett's T3 Test

	Control	DW15	DW25	WF15	WF25
Moisture	73.3 ± 0.32	72.2 ± 0.24	72.2 ± 0.25	72.0 ± 0.55	73.6 ± 0.41
Ash	6.6 ± 0.12^{a}	6.6 ± 0.10^{a}	6.8 ± 0.11^{ab}	6.8 ± 0.12^{ab}	7.1 ± 0.06^{b}
Protein	40.0 ± 0.18	39.7 ± 0.68	39.3 ± 0.34	39.4 ± 0.95	40.5 ± 1.31
Lipid	22.1 ± 0.36	22.0 ± 0.53	21.0 ± 0.41	21.5 ± 0.83	20.4 ± 0.99
HSI[1]	2.10 ± 0.37^{ab}	2.50 ± 0.59^{a}	1.88 ± 0.24^{b}	2.02 ± 0.44^{ab}	1.80 ± 0.30^{b}
ISI[2]	5.19 ± 0.76	5.14 ± 0.81	5.37 ± 0.85	4.97 ± 0.88	5.51 ± 0.84

[1]Hepatosomatic Index (HSI) = [Liver mass (g) / body mass (g)] × 100.

[2]Intestinal somatic Index (ISI) = [Intestine mass (g) / body mass (g)] × 100.

No striking differences in fat accumulation between the intestinal coils were observed upon dissection. Histology of the sac-like stomach is illustrated in Figure 5. The digestive tract exhibited the typical four-layered structure comprising a folded mucosa, the submucosa, the muscularis and the serosa, which is typically found in vertebrates. The strongly folded mucosa consisted of a single-layered columnar epithelium with interspersed mucus-secreting goblet cells facing the lumen of the stomach. A muscularis mucosa separating the lamina propria from the submucosa was not observed here. Forming a connective tissue core with blood vessels, the thin submucosa extended into the folds, thereby supporting the mucosa. Two prominent layers of striated muscle (inner longitudinal, outer circular) formed the muscularis, which was succeeded by the serosa. Fish fed experimental diets had a similar appearance when compared to the control. Consequently, diets did not affect the morphology of the stomach.

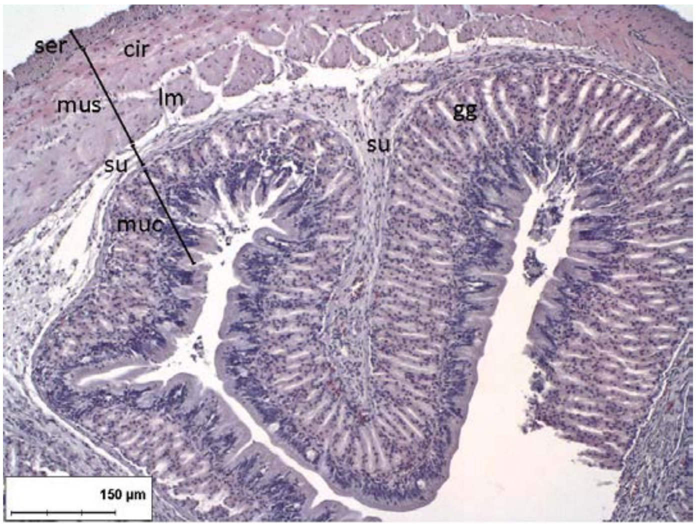

Figure 5: Histology of the sac-like stomach of Tilapia revealing no differences in fish fed control and experimental diets. Serosa (Se), muscularis (mus), longitudinal muscle (lm), circular muscle (cm), submucosa (su), mucosa (muc). Scale bars: 150µm. (H&E).

Macroscopically, the hepatopancreas in all fish were light brown (Figure 6), not indicating severe fattening as suggested by pale coloration in other studies, irrespective of diet fed. Histopathology of the liver did not reveal major abnormalities, neither in the control diets nor in the fish fed experimental diets. Hepatocytes were arranged in a typical parenchyma and pancratic tissue was evenly scattered within the liver tissue in close proximity to the blood vessels. Sinoids were irregularly distributed between the polygonal hepatocytes without any abnormalities such as congestion of sinoids. Congruently, yellow ceroid pigments, indicating nutritional stress, were rarely observed. Again, no difference between dietary groups was observed.

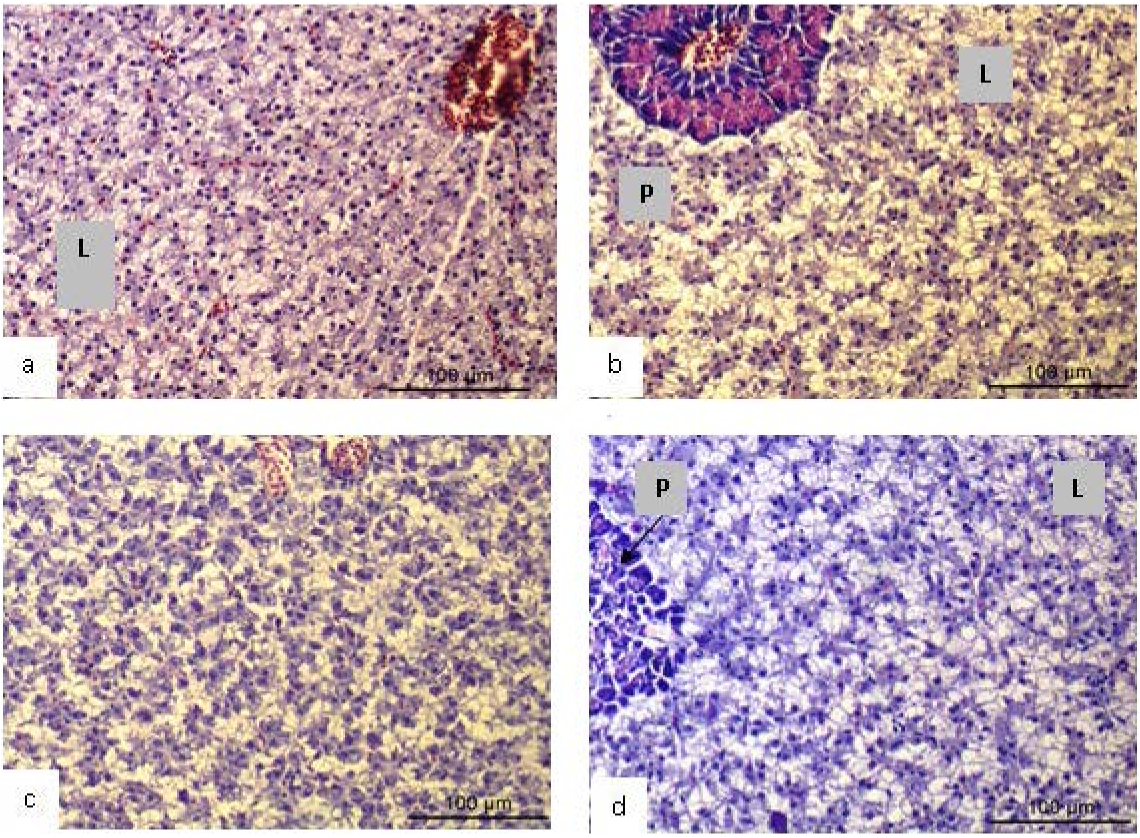

Figure 6: Histological sections of the hepatopancreas of Nile Tilapia in control fish group (a), and fish fed experimental diets DW15 (b), WF15 (c) and WF25 (d). Exogenous pancreas (P) embedded in liver tissue (L). Scale bars: 100µm. (HE-stained).

Discussion

The digestive system of Tilapias is relatively simple, comprising of a short oesophagus, a small sack-like stomach, and a very long, coiled intestine, which can reach 7 to 13 times bigger in length compared to the total fish length (Beveridge and Baird, 2000). This long digestive system indicates the herbivorous feeding behaviour of Tilapias. Therefore, this study focused on the utilization of plant material as feed sources. El-Sayed (1999) has reviewed this topic and has reported the potential of terrestrial plants (oilseed plants, grain legumes and plant concentrate proteins, and aquatic plants) as dietary protein sources for Tilapias. Although plant material results in a low biological performance compared to standard fish diets, he has recommended its use as fish feed due to its economical efficiency.

In Colombia aquatic plants have a high potential as fish feed since they are not used as food and they are greatly available in rural regions throughout the whole year. The nutritive value and potential of dried aquatic macrophytes as plant protein for fish feed has been examined in numerous studies (Fasakin et al, 1999; Kalita et al, 2007 and Abdel-Tawwab, 2008). It was reported that an inclusion level up to 25 % supported fish growth, when fish meal content ranged between 7.5 to 22.0 % in the diet. More recently, Cruz et al. (2011) evaluated the nutritional potential of sundried and fermented aquatic plants found in rural areas of Colombia. They recommended the used of fermented plant material as fermentation reduced the content of antinutrients and fibre.

Studies on evaluating other aquatic macrophytes as feed ingredients for Nile Tilapia reported similar or even lower SGR than those reported in this study. El-Sayed (2003) reported SGR of 2.9 and 2.8 (%day-1) for fish fed on diets (35 % CP and 38 % FM inclusion level) containing 20 % of molasses-fermented and yeast-fermented water hyacinth (*Eichornia crassipes*), respectively. In our study, with comparable CP, but lower FM inclusion (3% FM), a SGR of 2.8 and 2.9 (%day-1) was achieved at 25 % and 15 % fermented macrophytes inclusion. Nevertheless, Abdel-Tawwab (2008) reported a lowest SGR of 0.8 (%day-1) for Nile Tilapia fed on diets supplemented with sundried *Azolla pinnata* at 25 %. In his study diets contained 20 % CP and 10% FM inclusion level.

Unconventional plant protein sources indeed limit fish growth performance. In the literature, many factors are enumerated to explain this effect: Among the most important are the factors of reduced protein content, the unbalanced amino acid profile, the high fibre and ash content, and the presence of antinutritional substances. In this respect, the tested aquatic macrophytes showed moderate protein contents (241 - 264 g kg-1CP) compared to standard commercial plant ingredients. However, the amino acid profile of DW and WF seems to fulfil the requirements for lysine and methionine of common cultured tropical fish (Cruz et al, 2011).
To avoid adverse effects of antinutritional substances, the tested aquatic macrophytes were fermented. As Cruz et al. (2011) showed, trypsin inhibitor, phytates, soluble and condensed tannins, and oxalates were tremendously diminished.

Since fibre content in DW25 (133.7 g kg-1) and WF25 (137.2 g kg-1) diets was notably higher than in the control diet (68.2 g kg-1) overall digestibility might have been reduced in comparison to the control diet. In the past, increased fibre content of diets containing plant ingredients have shown to negatively affect weight gain, growth response, and protein utilization of Nile Tilapia (Omoregie and Ogbemudia 1993; Fagbenro et al. 2004). Also, Anderson et al. (1984) reported that a fibre level above 100 g kg-1 reduced feed efficiency and nutrient digestibility of Nile Tilapia causing poor fish growth. Here, a high fibre content (over 13 %) at 25 % DW and WF revealed significantly reduced dry matter.

In fact, the significantly higher FCR (2.5) in WF25 diets when compared to FCR (1.8) in control diets can be attributed to the high dietary fibre and ash content of the WF25 diets. An even higher FCR (4.2) was reported by Abdel-Tawwab (2008) for Nile Tilapia fed on diets supplemented with sundried WF (*Azolla pinnata)* at 25% inclusion level. In contrast, El-Sayed (2003) reported lower FCR (from 1.6 to 1.8) for fish fed on diets containing 20 % of molasses-fermented and yeast-fermented water hyacinth, suggesting a better FCR of fermented ingredients.

Increased dry matter, ash content as well as protein digestibility revealed that feed utilization in DW15, DW25 and WF15 was comparable to the control, but was substantially reduced in WF25. Interestingly, ADC of lipids was significantly lower in diets containing higher levels of both aquatic macrophytes and particularly in those containing WF. This could possibly be explained by the low lipid content (31.0 g kg-1) of aquatic macrophytes which could have also affected growth and feed utilisation as a result of the reduction of available dietary energy and deficiency of essential fatty acids. Otherwise, lipid body composition of Nile Tilapia fed the test diets was not significantly affected by dietary treatments. This coincides with El-Sayed (2003) findings evaluating fermented water hyacinth for Nile Tilapia. Even so, the level of whole body ash was significantly higher in fish fed on WF25 diets compared to the control group, which may be attributed to the particularly high ash content in WF.

Differences between the ISI values were not found between the diets. However, the HSI was significantly lower in fish fed on DW25 and WF25. This could be attributed to the significantly lower deposits of fat in these groups directly affecting the size of the liver, and could be furthermore explained by the lowest ADC of lipids found in the diets containing higher levels of macrophytes. Tusche et al. (2011) also referred to this observation and reported it as a sign of the effects of short-term starvation on fish hepatocytes.

In conclusion, fermented DW up to 25% and WF up to 15 % can be utilised in low-fish meal diets to reduce feeding costs without an impact on growth performance, feed conversion and animal welfare.

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CHAPTER 5.

On-farm evaluation of Cachama blanca and Nile tilapia fed fermented aquatic plants in a polyculture

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Abstract

Juveniles of Cachama blanca and Nile tilapia averaging 86.7 g and 39.6 g, respectively, were co-stocked in 12 experimental units (18 m^2 in area) at a total density of three fish m^{-2}.The species mixture consisted of 25% Cachama blanca and 75% tilapia. The fish were fed with a commercial aquafeed (24% crude protein) as control diet. Fermented duckweeds (DW), *Lemna minor* and *Spirodela polyrhiza*, and fermented water fern (WF), *Azolla filiculoides*, at 15% substitution level of the commercial aqua feed were offered as experimental diets for a total of three treatments with four replicates per treatment. The experiment was carried out during a period of 120 days of rearing. Growth performance and productive parameters were evaluated. Results on Cachama blanca showed that no significant differences were found for FCR (from 2.7 to 3.0), total Biomass (5536 to 6444 g) and yield (from 3076 to 3580 kg/ha) among treatments. Mean final weight (from 423 to 494 g), weight gain and specific growth ratio (SGR) were higher for Cachama blanca fed on control and DW diets than for those fed on WF diets. Similar results were observed for Nile tilapia. Mean final weight, weight gain and SGR of Nile tilapia fed on DW do not differed significantly from the control diet, but for those fed on WF, which performance was significantly lower. Yield for Nile tilapia varied from 3124 to 3829 kg ha^{-1} and did not differ between the treatments. Average FCR was calculated for Nile tilapia between 2.1 and 2.5 and was better than for Cachama blanca. Results indicated that fermented duckweeds (*Lemna minor* and *Spirodela polyrhiza*) can effectively replace commercial aqua feed at a 15% level for rearing juveniles of Cachama blanca and Nile tilapia in a traditional polyculture.

Key Words: *Cachama, duckweeds, fermentation, polyculture, tilapia, water fern.*

Resumen

Juveniles de Cachama blanca y Tilapia del Nilo con un promedio de 86.7 y 39.6 g, respectivamente, fueron cultivados en 12 unidades experimentales (18 m^2) con una densidad total de tres peces m^2. La mezcla de especies consistió en 25% Cachama blanca y el 75% Tilapia. Los peces fueron alimentados con un pienso comercial (proteína bruta 24%) como dieta de control. La lenteja de agua fermentada (Duckweeds: DW), (*Lemna minor* y *Spirodela polyrhiza*), y la *Azolla fermentada* (Water fern: WF), (*Azolla filiculoides*), fueron ofrecidas como dietas experimentales al 15% de nivel de sustitución para un total de tres tratamientos con cuatro repeticiones por tratamiento. El experimento se llevó a cabo en un período de 120 días. El crecimiento y los parámetros productivos fueron evaluados. Los resultados de

Cachama blanca mostraron que no se encontraron diferencias significativas para el FCR (2,7-3,0), la biomasa total (desde 5536 hasta 6444 g) y el rendimiento (3076 a 3580 kg/ha) entre los tratamientos. El peso final (423-494 g), la ganancia de peso y la tasa de crecimiento específico (SGR) fueron mayores para la Cachama blanca alimentada con la dieta control y la dieta DW que para aquellos alimentados con la dieta WF. Resultados similares fueron observados para la Tilapia del Nilo. El peso final medio, la ganancia de peso y la tasa de crecimiento específica (SGR) de la Tilapia del Nilo alimentados con la dieta DW no diferían de manera significativa de la dieta control, pero sí de los peces alimentados con la dieta WF, lo cuales mostraron un desarrollo significativamente menor. El rendimiento de la Tilapia del Nilo varió de 3124 a 3829 kg/ha y no hubo diferencias entre los tratamientos. El FCR promedio calculado para la Tilapia del Nilo fue entre 2.1 y 2.5 y resultó mejor que para la Cachama blanca. Los resultados indicaron que la lenteja de agua fermentada (*Lemna minor* y *Spirodela polyrhiza*) puede reemplazar efectivamente hasta un 15% del alimento comercial en un policultivo tradicional de Tilapia del Nilo y Cachama blanca.

Palabras clave: *Cachama, lenteja de agua, fermentación, policultivo, Tilapia, Azolla.*

Resumo

Juvenis de Pirapitinga e Tilápia do Nilo com uma média de 86.7 e 39.6 g, respectivamente, foram cultivadas em 12 unidades experimentais (18 m^2) com uma densidade global de três peixes m^2. A mistura de espécies consistiu de 25% de Pirapitinga e 75% Tilápia do Nilo. Os peixes foram alimentados com uma ração comercial (24% de proteína bruta) como dieta de controle. As lentilhas da água fermentada (duckweeds: DW), *Lemna minor* e *Spirodela polyrhiza*, e Azolla fermentada (water fern: WF), *Azolla filiculoides*, foram oferecidas como dietas experimentais a 15% de nível de substituição para um total três tratamentos com quatro repetições por tratamento. O experimento foi realizado durante um período de 120 dias. Foram avaliados os parâmetros de crescimento e de produção. Resultados para a Pirapitinga não mostraram diferenças significativas para o FCR (2.7-3.0), a biomassa total (5536-6444 g) e rendimento (3.076-3.580 kg/ha) entre os tratamentos. O peso final (423-494 g), ganho de peso e taxa de crescimento específico (TCE) foram maiores para a Pirapitinga alimentada com a dieta controle e dieta DW do que para aqueles alimentados com a dieta WF. Resultados similares foram observados para a Tilápia do Nilo. O peso médio final, ganho de peso e taxa de crescimento específico (TCE) da Tilápia do Nilo alimentada com a dieta DW não diferiu significativamente da dieta controle, mas, sim, daqueles peixes alimentados com dieta WF,

que mostraram significativamente menos desenvolvimento. O desempenho da Tilápia do Nilo variou de 3124-3829 kg/ha e não houve diferenças entre os tratamentos. A FCR média calculada para a Tilápia do Nilo foi dentre 2.1 e 2.5 e foi melhor do que para a Pirapitinga. Os resultados indicaram que a lentilha de água fermentada (*Lemna minor* e *Spirodela polyrhiza*) pode efetivamente substituir até 15% do alimento comercial em uma policultura tradicional de Tilápia do Nilo e Pirapitinga.

Palavras-chave: *Pirapitinga, lentilha d'água, fermentação, policultura, Tilápia, Azolla.*

Introduction

According to FAO (2012) the major aquaculture producing countries of Latin America in 2010 were Chile (701.062 tonnes), Brazil (479.399 tonnes), Ecuador (271.919 tonnes), Mexico (126.240 tonnes), Peru (89.021 tonnes) and Colombia (80.367 tonnes). Florez-Nava (2007) reported that the aquaculture sector in the region is mainly based on tropical and subtropical species (excepting Chile) and export-oriented, therefore it is highly depending on fish feed or pelleted diets. Fishmeal constitutes the main raw material for fish feed. However, due to the high demand and cost of fishmeal, its substitution with locally available vegetable proteins has become prevalent in the region, mainly in the rural sector.

A variety of aquatic plants are widely available thought the whole year in several water bodies in the tropics. Some of them have a good nutritional composition and are already used in the diet of monogastric animals. However, many of them remain still underused. The Duckweeds (*Lemna minor* and *Spirodelapolyrhiza*) as well as the Water fern (*Azollafiliculoides*) possess high protein content (15.7% to 26.4% CP) and have been tested for acceptability and digestibility in previous experiments with tropical fish species (Cruz et al, 2011b). Thus, their use seems be suitable for replacement of fishmeal in the diet of omnivorous-herbivorous fish.

Among the commonly farming fish cultivated in Latin America the characids are the most important group of native fish. The Tambaqui (*Colossomama cropomum*), the Pacu (*Piaractus mesopotamicus*) and the Cachamablanca (*Piaractus brachypomus*) are widely cultured in their native river basins. The Tambaqui and Pacu have also been introduced to other countries within and outside the region (FAO, 2005), whereas Cachama blanca has become economically the most important fish in Colombia (Espinal *et al.*, 2005).

Considering that cultivation of different fish species in the same pond makes feasible the optimum utilization of the available resources in the aquatic environment, the polyculture of tilapia, the most common tropical farming fish, and a native species as the Cachama blanca (*Piaractus brachypomus)*seems to be a good alternative to increase the productivity of the rural tropical aquaculture. Moreover, the integration of these species in a polyculture is highly according to the recent efforts dedicated to the utilization of native species in South America.

The Nile tilapia (*Oreochromis niloticus*) is an omnivorous and filtrating fish that feeds plant material, phytoplankton and benthos by preference. Cachama blanca (*Piaractus brachypomus*) feeds primarily on plant material. Consequently, the provision of primary food through fertilizing the ponds and through use of aquatic plants that require minimal effort to be cultivated has the potential to lead to economic feasibility for small-scale farmers. These species, Tilapia and Cachama blanca, are considered a very effective food fish.

Thus, the objective of this study was to evaluate the effect of replacing 15% commercial fish feed (24% crude protein) with fermenting locally available aquatic plants; duckweeds *Lemna minor* and *Spirodela polyrhiza*(DW15) and the water fern *Azolla filiculoides* (WF15), on the growth performance and productive parameters of two valuable fish species, Tilapia and Cachama blanca, in a traditional polyculture.

Material and Methods

This study was conducted from June to October 2009 using the facilities of Instituto de Investigaciones Tropicales (Intropic) at the University of Magdalena in Colombia (longitude 74º 07' and 74º 12' W - latitude 11º 11' and 11º 15' N). The experiment was carried out for 120 days. Three treatment diets were tested each with four replicates.

Culture system and stocking of fish

The fish used in this study, Nile tilapia (*Oreochromis niloticus*) and Cachama blanca (*Piaractus brachypomus*), were obtained from local suppliers in Villavicencio (Colombia) and remained in the Aquaculture Laboratory of the University of Magdalena until all juveniles of Nile tilapia and the Cachama blanca achieved a weight averaging 39.6 ± 2.52 g and 86.7 ± 4.5 g (mean ± SD), respectively.

Juveniles were cultivated in one earth pond divided into 12 experimental units, each one with 18 m^2 in area and 0.9 m in depth. The stocking ratio of fish was distributed to the proportion 75% Nile tilapia and 25% Cachama blanca with a total density of three fish per m^{-2}. Before stocking of the culture pond a careful selection of only male Nile tilapia was determined through manual sexing. The pond was prepared prior through draining and sun drying. Afterwards, it was fertilized and then filled with water until the level reached 0.9 m. Fertilization consisted of 400 kg ha^{-1} poultry manure inserted weekly during the first month. In order to maintain the plankton population, fertilization was carried out monthly using the water transparency as a paramater (Secchi-disk). The pond was refilled with water once per week to replenish infiltration and evaporation.

Feed and feeding regime

As a control diet, fish were fed on a 24% protein commercial aqua feed. The experimental diets were composed of 85% commercial aqua feed and 15% of each of the tested ingredients; the duckweeds *Spirodela polyrhiza* and *Lemna minor* (DW15)and the water fern *Azolla filiculoides* (WF15) treated by lactic acid fermentation. The fermentation of aquatic macrophytes was carried out as described by Cruz et al. (2011a) through the supplementation with LAB inoculants and molasses. The dietary ingredients were ground, mixed homogeneously and dry pelleted to 4 mm diameter. Diets were offered at 4% of mean fish biomass 6 days per week and at two times per day (10:00 and 17:00). During the trial, the feeding rate was reduced gradually to 2% of biomass. The analysis of the nutritional composition for the diets was performed according to AOAC (2005) and is shown in Table 5.1.

Table 5.1: Composition (%) and nutrient content (g kg^{-1}) of the commercial feed diet and the experimental diets on dried matter basis. CD: Commercial diet (CD); DW15: Duckweeds at 15% inclusion level; WF15: Water fern at 15% inclusion level.

Ingredients (%)	CD	DW15	WF15
Commercial fish diet[1]	100	85	85
Fermented Duckweeds (*Lemna* and *Spirodela*)	0	15	0
Fermented Water fern (*Azolla*)	0	0	15
Total	100	100	100
Nutrient content (g kg^{-1})	CD	DW15	WF15
Dry matter	880	888	887
Crude protein	240	243	241
Lipids	60	56	56
Fibre	80	85	83
Ash	120	142	153

[1] Obtained from ITALCOL Alimentos Concentrados © (Villavicencio, Colombia)

Growth and yield

Fish were weighed and measured monthly for growth determination and feed adjustment. At least 10% of each species of the fish population were collected from each experimental unit. The total fish weight was calculated using the mean weight of the sampled fish multiplied by the total number of fish stocked at the beginning of the trial. After 120 days fish were harvested, the data was collected for total weight, size, and number of fish and the survival rate, feed conversion and yield were determined.

Growth, yield, feed utilization and survival parameters measured or calculated were the following: *initial* (${}_{i}W$) *and final* (${}_{f}W$) *mean body weight* (g), *total biomass* (g), s*pecific growth rate* (SGR) = $[((\ln {}_{f}W - \ln {}_{i}W)/120 \text{ days}) \times 100]$; *weight gain* (WG) = $[{}_{f}W - {}_{i}W]$; *per cent weight gain %* (PWG)= $100 \times ({}_{f}W - {}_{i}W)/{}_{i}W$; f*eed conversion ratio* (FCR) = [total feed intake in dry basis (g)/wet weight gain (g)]; *feed conversion efficiency %* (FCE)= Weight gain (g) x100 / weight of feed given; *survival rate* (SR) = [(total number of fish harvested x 100)/total number of fish stocked] and *yield* (Y) = [total number of fish stocked x SR x WG (kg)/area of the ponds (ha)].

Water quality

The water quality parameters were evaluated daily during the 120 day trial. Dissolved oxygen and water temperatures were measured early in the morning and in the afternoon using an oxygen sensor (Hanna HI9146). The pH (Hanna hand-pH-meter) was also measured. Total ammonia-N was measured weekly using a colorimetric method. Total hardness was measured every 15 days using a colorimetric test.

Statistical analysis

Data are presented as treatment means ± SE (standard error). Results of the three groups were analysed using one-way analysis of variance (ANOVA). Means of statistically different variables were compared by Tukey's test and LSD test ($P < 0.05$). Homogeneity of the variances was determined by Levene's Test ($P < 0.05$) and by Dunnett`s T3 ($P < 0.05$) for homogeneous and inhomogeneous variances, respectively. All data were statistically analysed using SPSS (version 19) software package for Windows.

Results

The composition of the experimental diets is given in Table 5.1. In the diet composition a significant variation can be observed in the ash content. Control diet (CD) presents an ash content of 120 g Kg^{-1}, while diets including fermented water plants show a higher level of ash. In the case of DW15, ash content is 18% higher compared to the control. This difference is even more drastic for WF15 with 28% higher ash content compared with the control. Water quality parameters of the experimental units are presented in Table 5.2. Temperature and pH were constant and presented an average of 28.5 °C and 7.6, respectively. The minimal values of the dissolved oxygen (DO) were registered in the early morning ranging from 2.0 to 2.6 mg l^{-1}. During the day DO varied into optimal ranges from 6.7 to 8.3 mg l^{-1}. The values of total ammonia-N did not present high variations, due to the fact that a constant refill was maintained.

Table 5.2. Water quality parameters (means ± SD) in the experimental pond.

Parameters	Experimental Earth Pond
Temperature (°C)	28.5 ± 0.98
Dissolved oxygen (mg l^{-1}), early in the morning	2.3 ± 0.22
Dissolved oxygen (mg l^{-1}), after midday	7.5 ± 0.51
pH	7.6 ± 0.36
Total ammonia-N (mg l^{-1})	0.074 ± 0.014
Total hardness (mg l^{-1} CaCO3)	162.7 ± 10.53

Growth performance, feed efficiency and production parameters of Cachama blanca and Nile tilapia in polyculture are shown in Table 5.3. Final mean weight of Cachama blanca ranged from 422.8 to 494.1 g, percent of weight gain of Cachama blanca varied from 373 to 497% and SGR from 1.29 to 1.49 % day^{-1}, indicating in all cases significant differences ($P < 0.05$) among control and WF15 treatment. Weight gain of Cachama blanca among treatments was similar during the first 60 days (Figure 8). Afterwards, Cachama blanca fed on control and DW15 diets gained weight faster than fish fed on WF15. This result in weight gain of Cachama blanca did not differ significantly between fish fed on control diet and DW15 (Figure 7), but did between control and WF15 (Figure 8). FCR varied from 2.7 to 3.0 and no differences ($P > 0.05$) were found. Survival of Cacha mablanca in the polyculture was over 72%. Total biomass and yield of Cachama blanca was also expected to be significantly affected by treatments, however no significant variations were found. Although the differences were not significant, probably due to variability between replicates, they may be considered relevant. Yield of Cachama blanca fed on WF15 was about 14% lower than for control and DW15.

Table 5.3. Growth performance, feed efficiency and production parameters (means ± SE) of Cachama blanca (*Piaractus brachypomus*) and Nile tilapia (*Oreochromis niloticus*) stocked in pond. Values with a different superscript in the same row (fish species separately) are significantly different ($p < 0.05$).

	Cachama blanca (*P. brachypomus*)			Nile tilapia (*O. niloticus*)		
	CD	DW15	WF15	CD	DW15	WF15
Initial weight (g)	82,8 ± 1,06	87,9 ± 2,52	89,3 ± 1,93	38,1 ± 1,91	40,6 ± 0,30	40,1 ± 0,96
Final weight (g)	494,1 ± 11,38 ^{a}	473,4 ± 22,07 ^{ab}	422,8 ± 20,65 ^{b}	229,8 ± 2,60 ^{a}	249,70 ± 9,54 ^{a}	187,8 ± 11,68 ^{b}
Weight gain (g)	411,3 ± 12,10 ^{a}	385,5 ± 21,57 ^{ab}	333,5 ± 19,70 ^{b}	191,8 ± 3,99 ^{a}	209,1 ± 9,43 ^{a}	147,7 ± 12,45 ^{b}
Percent weight gain (%)	497 ± 19,5 ^{a}	493 ± 26,4 ^{ab}	373 ± 19,4 ^{b}	509 ± 37,0 ^{a}	515 ± 22,3 ^{a}	371 ± 37,4 ^{b}
Specific Growth Rate (%/d)	1,49 ± 0,03 ^{a}	1,40 ± 0,04 ^{ab}	1,29 ± 0,03 ^{b}	1,50 ± 0,04 ^{a}	1,53 ± 0,03^{a}	1,30 ± 0,07 ^{b}
FCR	2,7 ± 0,11	2,8 ± 0,17	3,0 ± 0,15	2.1± 0.09	2.3 ± 0.21	2.5 ± 0.20
FCE	37.4 ± 1.1	34.9 ± 1.6	32.8 ± 1.2	48.3 ± 2.6	44.0 ± 4.4	41.7 ± 4.0
Survival rate (%)	72,5 ± 2,25	74,0 ± 0,91	72,8 ± 3,33	83,3 ± 3,94	75,7 ± 7,28	84,0 ± 4,59
Total Biomass (g)	6444 ± 223,8	6297 ± 241,2	5536 ± 365,1	6893 ± 317,9	6759 ± 504,5	5677 ± 480,8
Yield (kg/ha)	3580 ± 124,3	3498 ± 134,0	3076 ± 202,8	3829 ± 176,6	3755 ± 280,3	3124 ± 267,1

nd: not determine

Commercial diet (CD) ; Duckweeds at 15% inclusion level (DW15); Water fern at 15% inclusion level (WF15)

Means with different letters in the same row for each species are significantly different ($P < 0.05$).

Specific growth rate (SGR) = [lnWf (mean final weight) − lnWi (mean initial weight)/No days] × 100.
Wi= initial weight, Wf = final weight

Percent weight gain (WG) = 100(Final weight-Initial weight)/ Initial weight.

Feed conversion ratio (FCR) = total feed intake in dry basis (g) / wet weight gain (g).

Feed conversion efficiency (FCE)= Weight gain (g) x100 / weight of feed given (g)

Similar results were obtained for Nile tilapia. Final mean weight ranged from 187.8 to 249.7 g, percent of weight gain varied from 371 to 509 %, and SGR varied from 1.3 to 1.5 % day^{-1} indicating significant differences ($P < 0.05$) among treatments. Weight gain of Nile tilapia during treatments was similar during the first 90 days (Figure 10). Afterwards, Nile tilapia fed on WF15 showed a significantly lower growth performance ($P > 0.05$). Nile tilapia fed on DW15 displayed a trend of higher weight gain than the control diet. Growth parameters of Nile tilapia fed on DW15 and on the control diet did not present differences, but fish fed on WF15 showed a significantly lower growth (Figure 9 and 10). FCR varied from 2.1 to 2.5 and no statistically differences ($P > 0.05$) were found. In the case of Nile tilapia survival was over 75% and did not vary ($P >0.05$) among treatments. Total biomass and yield did not vary significantly. However, yield of Nile tilapia fed on WF15 was around 18% lower than for control and DW15.

Calculated FCR and FCE for the polyculture system presented significant differences between control (2.4 and 41.6 %) and WF15 (2.8 and 36.0 %) treatment.

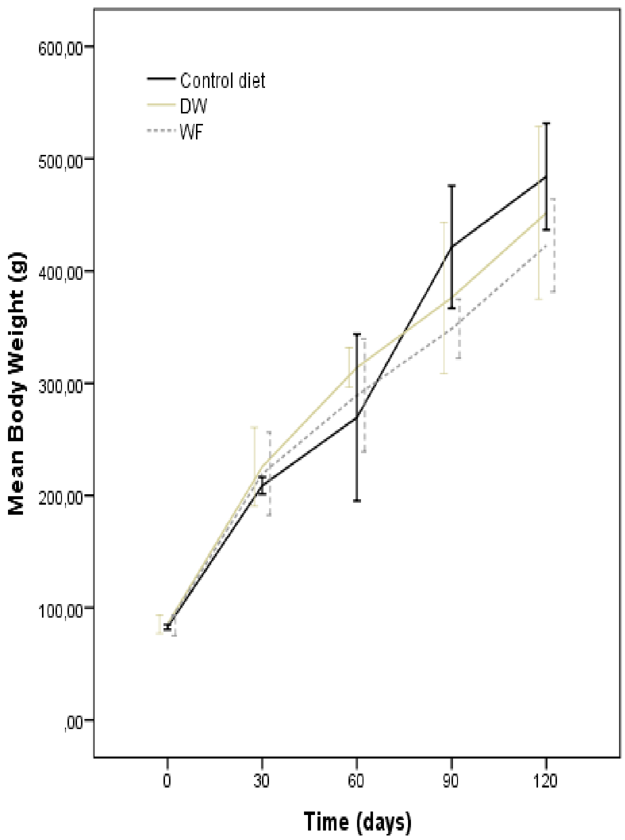

Figure 7. Mean body weight (g) of Cachama blanca juveniles (Piaractus brachypomus) rearing in polyculture with Nile tilapia. Values are means ± SE (n=4).

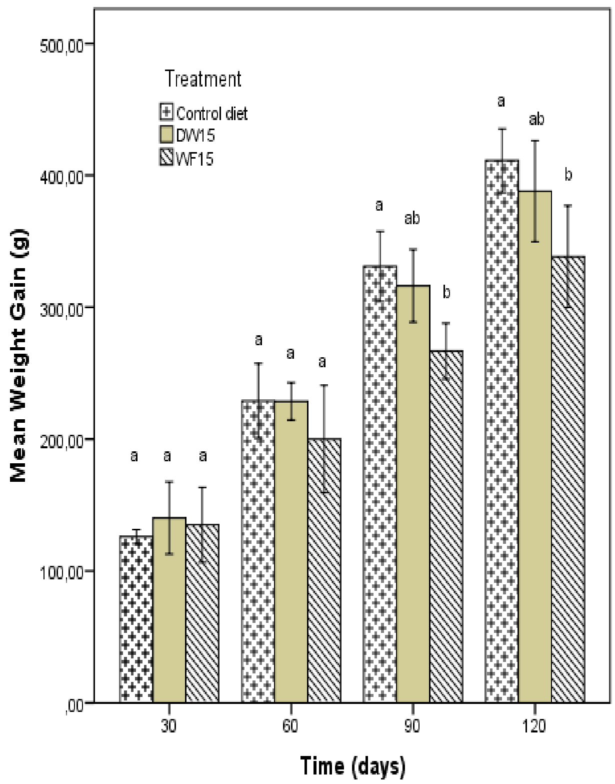

Figure 8. Mean weight gain (g/fish/month) of Cachama blanca juveniles (Piaractus brachypomus) rearing in polyculture with Nile tilapia.

Results in percentage of weight gain, SGR, survival rate, total biomass and yield for both species were comparable. Results showed that Cachama blanca and Nile tilapia grow well when fed on commercial diets replaced at 15% by DW, whereas the use of WF at the same inclusion level degraded growth performance of both species.

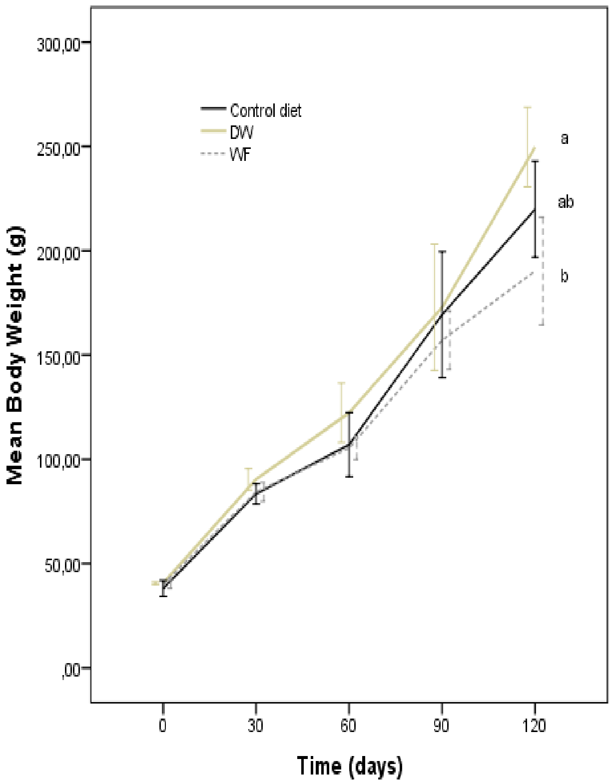

Figure 9. Mean body weight (g) of Nile tilapia juveniles (Oreochromis niloticus) rearing in polyculture with Cachama blanca. Values are means ± SE (n=4). Means with different letters are significantly different (P < 0.05).

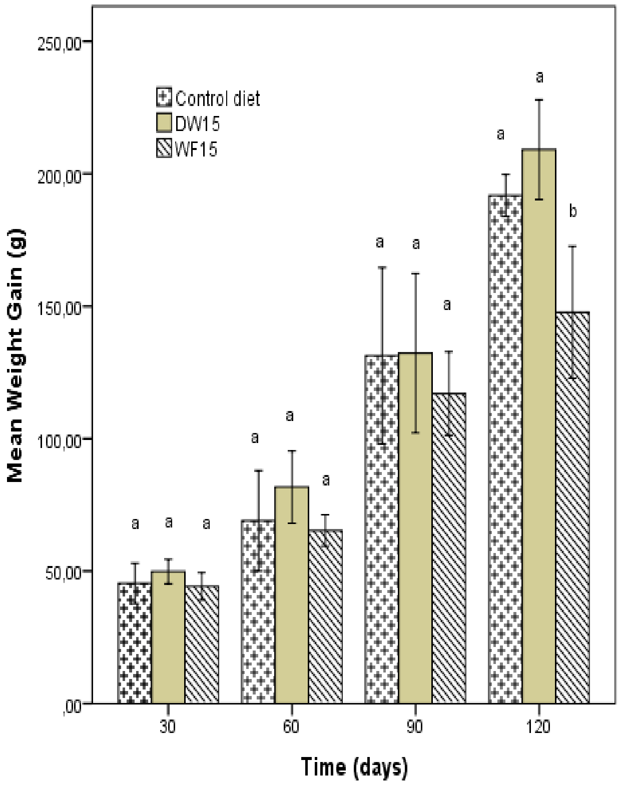

Figure 10. Mean weight gain (g/fish/month) Nile tilapia juveniles (*Oreochromis niloticus*) rearing in polyculture with Cachama blanca. Means with different letters are significantly different ($P < 0.05$).

Discussion

Climate conditions during the experimental period were marked for unusually higher rainfalls due to the climatic phenomena *La Niña* which occurs around the Pacific coast and leads to dramatic weather conditions in whole Colombia. Although climate affects water parameters, no variations were noticed in the water quality factors among the different treatments as all experimental units were placed in the same pond, adjacent to one another and receiving water from the same source. Water quality parameters in the pond ranged within the acceptable requirements reported for cultured fish species (Boyd, 1982). Temperature and pH were found to be the more consistent parameters during the course of the trial. Oxygen was marked lower in the early morning, but it did not affect feed consumption when feed was offered around 10 am.

A comparison between the two supplemented water plants shows that their inclusion into the diets affected the utilization of nutrients by Cachama blanca and Nile tilapia. Growth was significantly reduced when WF was included at 15%. Rather than from the beginning of the experimental period, it was determined that growth was affected only after a period of time which suggests a progressive accumulation of one or more elements with an anti-nutritional effect. A potential explanation was the high ash content in WF15 diet, Cruz et al. (2011) reporting that mineral concentration should be considered before the inclusion of water fern *Azolla filiculoides* into fish diets due to its high content of zinc, copper and chromium. For confirmation of this result further study on carcass composition must be carried out. Otherwise, fish grew well when duckweeds were included at 15%, for Cachama blanca fed on DW15 there were no significant differences with the control and for Nile tilapia fed on DW15 growth performance was better than in the control, but no significant.

This result was observed in a previous experiment with Nile tilapia. According to Cruz Velázquez et al. (2015) Nile tilapia tolerates inclusion of DW up to 25% in formulated diets (35% CP) with low fish meal content, while for the same diet the maximum level of WF was 15% without an impact on growth performance, feed conversion and animal welfare

Final weight gain of Cachama blanca was two-fold higher than for Nile tilapia. However, percentage of weight gain was very close and comparable between the two species. In fact, in the trend, the percentage of weight gain was higher for Nile tilapia. It was not expected. A relative restricted growth and feed utilization by Nile tilapia comparable to Cachama blanca was assumed due to the early breeding of female Nile tilapia mistakenly included in the population, which occurred despite manual sexing being carried out. Otherwise, the comparable growth percentage of Nile tilapia could be due to its feeding on plankton from natural pond biota, in contrast to Cachama blanca which is a non-filter feeder fish that is unable to take advantage of the plankton. Therefore, Nile tilapia could compensate its energy requirements. Green et al. (1994) reported in their study that Tilapia grows adequately in fertilized ponds with only natural productivity. Also the extensively stocked ponds (3 fish m^{-2}) could have a positive impact on the recovery of the energy balance for Nile tilapia, especially during the last phase of the experimental period. Fish growth is highly dependent on stocking density (Jha and Barat, 2005). An increasing density is accompanied by an increase on stress, feed competition and therefore energy requirements which lead to a reduced growth and feed utilization (Pickering, 1993, Abou Y. et al, 2007).

Tilapia reproduction occurred commonly as a result of imperfect sex reversal. This problem is commonly avoided by the careful selection of only males prior to the stocking of the culture pond, as done in this study and alternatively predatory fish are carefully utilized to thin out the Tilapia (Green et al, 1994). It had been assumed that females of Nile tilapia used large percentage of the ingested energy for the physiological changes associated with sexual maturation affecting the maximum attainable size of the original stock in grow out. Therefore small fish were harvested monthly in order to maintain the equilibrium of the culture.

The SGR values obtained for Cachama blanca in this study was comparable to those reported by Vásquez et al. (2012) in their study on Cachama blanca in cages (SGR from 1.38 to 1.46 % day^{-1}) and in ponds (1.33 to 1.70% day^{-1}) offering formulated diets at 26, 30 and 34% of digestible protein level. In their study, Vásquez et al. (2012) obtained the best SGR from diets with the maximal protein level, whereas in the present study protein level in all diets was 24%. These results confirm that in captivity Cachama blanca accepted very well artificial diets even with low levels of protein as reported by Vasquez-Torres (2005). In another study, Tafur-Gonzales et al. (2009) also presented SGR (1.75 % day^{-1}) and weight gain (449.7 g) values for *P. brachypomus* raised in polyculture with the planctivore cichlid *Chaetobranchus semifasciatus*. These results, as well as ours, confirm the significant growth performance of Cachama blanca in polyculture. This has also been reported by Ascón et al. (2003) in their study on polyculture of Cachama negra (*Colossoma macropomum*) and Boquichico (*Prochilodus nigricans*). Several authors reported that *P. brachypomus* and *Colossoma macropomum* normally reach a weight from 300 to 600 g between 4 and 6 months of culture (Campos-Baca and Kohler, 2005; Lochmann et al, 2009), which is in accordance with the results obtained in this study.

The FCR for Cachama blanca was higher than those reported in the literature for the polyculture of this species. It is possible that the overpopulation of Nile tilapia also affected the feed consumption of Cachama blanca. In general there are limited number of reports in the literature regarding the culture of Cachama blanca in earthen ponds and its productive parameters. Tafur-Gonzales et al. (2009) reported a FCR of 1.05, which is very good, but in their study, total density of fish was also much lower (1 fish m^{-2}). Abimorad et al. (2009) reported FCR from 1.35 to 1.85 in diets for Pacú (*Piaractus mesopotamicus*) reared in cages and fed on diets based on plant ingredients and supplemented with lysine and methionine, whereas the basal diet without dietary supplementation showed a FCR of 2.15, which is closest to the results of this study. Vásquez and Arias (2012) also reported FCR of 1.0 for Cachama blanca, when it was fed on a semi purified diet containing three different sources of carbohydrates and lipids at 32% protein level.

Yield parameters were comparable among treatments and they were similar to those reported by other authors for the polyculture of Nile tilapia and characids as Cachama blanca. Teichert-Coddington (1996) reported a comparable mean production from 2537 to 5265 kg/ha in his study on polyculture of Nile tilapia and Cachama negra (*Colossoma macropomum)* in Central America. Tafur Gonsalez et al. (2009) also reported similar mean production (3825 Kg/ha/year) for *P. brachypomus* in polyculture with Cachama negra (*Colossoma macropomum*) and the cichlid *Chaetobranchus semifasciatus,* a cichlid herbivore, and native of the Amazon. Total biomass suggests that polyculture of Cachama blanca and Nile tilapia could result in two harvests per year with yields of aprox. 7000 and 7500 kg/ha/year, respectively, offering commercial feed (24% CP) supplemented with fermented DW at 15% and maintaining a constant production of plankton in the pond.

Results show that these two fish species are very well suited for production in extensive polyculture systems. In these systems, commercial diets with low protein content provide both good biomass and yields. The substitution with duckweeds appeared to have no negative affect on all the production parameters. It is therefore highly recommended as cheaper feed component in rural production. Water fern, which is easier to harvest, could be used for a limited time (max. 60 days) in the culture without causing a negative effect.

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This study discusses the effects of the use of aquatic plants in diets for tropical fish with herbivores-omnivorous habits in low income regions of Colombia. Aquatic plants are an excellent free source of nutrients, principally protein, widely distributed in rural areas around natural water bodies in the tropical regions. Aquaculture is one of the most important economic activities in rural areas and plays an important role for food security. A possible way to the successful development of the rural sector is the study of strategic issues related to rural aquaculture. In particular, the availability and quality of low-cost feed as well as the establishment of harmonized environmental strategies for a positive socio-economic impact of the aquaculture should be studied because the results are promising.

Aquatic Plants

-Why aquatic plants?

Although it is widely known that alternative nutrient sources produce lower biological performance of fish than commercial feeds based on fish meal, the reduction in fish performance would be compensated by the reduction of the costs of ingredients in the diets (El-Sayed, 1999). In a rural context it becomes more relevant, since aquaculture will be unable to depend on the costly fish meal as a major protein source. At the same time, the most research on the potential of alternative plant protein for fish feed has been focused on soybean and its sub-products, which have become as expensive as fish meal. Thus, the use of a freely available resource like the aquatic macrophytes will definitely decrease the cost of fish feeding by the partial replacement of fish meal or other ingredients in the diet.

Aquatic macrophytes do not require cultivation and their nutrient characteristics are highly variable, depending on the particular local growth conditions (Boyd, 1971). The results of this study (**Chapter 1**) showed that the nutritional composition of the raw aquatic macrophytes was characterized by a high ash (<14 g kg^{-1}) and crude fibre content (<10 g kg^{-1}), a limited lipid content (17-33 g kg^{-1}), an acceptable protein content (160-210 g kg^{-1} DM) considering the requirement of fish. The content of ash and fibre for the tested plants in this study was however comparable with values reported for several aquatic plants (Kalita, P. et al., 2008, Kalita, P. et al., 2007, El-Sayed, 2003, Ray & Das, 1995, Edwards, 1987).

Beside their valuable nutrient content, the locally available aquatic plants also revealed a relatively adequate profile of amino acids: 5.3 to 6.3 g lysine for 100 g of protein and 1.7 to

2.0 g methionine for 100 g of protein. Lysine is the first limiting essential amino acid in most feedstuffs; therefore the dietary level of lysine can significantly affect the growth performance and health of farmed fish (Mai et al., 2006). Methionine is a sulphur containing amino acid and is also essential for fish, so it must be given through food because it cannot be synthesized by the body. This amino acid has an important role in protein synthesis and other physiological functions in fish, such as intermediate in the synthesis of cysteine and taurine (Wilson, 2002; Li et al., 2009). The experimental diets containing the aquatic plants met the nutritional requirements of the studied fish.

Aquatic macrophytes are rich in minerals, exceeding the fish requirements but not the critical values for fish. Mineral composition of the raw aquatic macrophytes revealed a comparable content among plants, except for Azolla, which showed the highest amount of minerals. Although it does not exceed the tolerable limit for fish diets reported in NRC (1993); mineral concentration should be considered before the inclusion of Azolla into fish diets, depending on the fish species and its particular tolerable limits. The heavy metal concentration is particularly important as aquatic plants tend to accumulate them. In this study the concentrations of heavy metals were not critical, but if aquatic macrophytes were used as exclusive feed source for fish, heavy metal contamination of feed, particularly cadmium retention, must be considered since it reduces fish growth, feed conversion and can be toxic.

-Why is the fermentation of the aquatic plants recommendable?

As has been mentioned above, the tested aquatic plants showed a high ash and fiber content. Also, aquatic plants as other plant materials contain anti-nutritional substances (such as trypsin inhibitor, oxalates, tannins, phytates) which can negatively affect feed digestion by fish as well as fish growth performance.

Aquatic plants can be used in three different ways in fish nutrition: as fresh material, as sun-dried material and as fermented material. In its fresh state it requires a large volume of available plants in short time to meet the requirements of fish. Sun drying causes a loss of many of the plant's nutritional properties and, additionally, the palatability decreases because the ash content increases. The fermented material on the other hand has two major benefits: The main benefit that it allows preserving the harvested plant material and it is suitable for a use over long periods of time. The second major benefit is that fermentation reduces the fiber and ash content with a consequent increase in the plant digestibility. Therefore, fermented aquatic plants are far more digestible.

In the experimental diets (**Chapter 2**), the protein digestibility coefficients of the aquatic plants for *Piaractus brachypomus* was significantly higher ($P < 0.05$) when diets containing the fermented plant material were offered. Apparent digestibility of protein varied between 74.9% and 84.5% for fermented aquatic plants and between 51.1% and 60.4% for sundried aquatic plants. The combination of duckweeds, *Spirodela polyrhiza* and *Lemna minor*, showed a higher digestibility than the water fern *Azolla filiculoides*. The digestibility coefficients of energy did not reveal significant differences among treatments.

Beside the reduced fibre and ash content of the plants, fermentation reduced the content of undesirable substances; the concentration of antinutrients was considerable lower in the fermented material than in the fresh plant material (Cruz et al., 2011). In general, the concentration of common antinutrients as trypsin inhibitor, oxalates, phytates and soluble and condensated tannins in the aquatic plants did not exceed the tolerable limits reported for fish.
An interesting feature of the aquatic plants was that despite their high buffer capacity (between 70 to 90 $g.kg^{-1}$ lactic acid), low content of soluble sugar (<10 $g.kg^{-1}$) and the high moisture content (>90%), the lactic acid fermentation of the selected plants resulted in silages with a very good quality (>90 points of DLG) through the reduction of the moisture content (<50%) and the use of additives: bacterial inoculants (source for Lactobacillus) and molasses (source for soluble carbohydrates).

Experimental Diets

-Why should fish meal be replaced?

The use of fish meal for fish feed in the aquaculture industry has increased the environmental concerns about the depletion of the fish stocks of the oceans for its use as feed (fish meal and fish oil) for farmed fish. Nearly a third of the fish caught in the world is not used for human consumption, but to produce feed for other fish (Delgado et al., 2003). About 88.5% of the fish oil and 65.8% of the fishmeal produced globally is used for formulated diets for fish (Tacon and Metian, 2008). For years there have been efforts to find alternative diets based on plant sources such as corn, soybeans, cotton, peanut and wheat. Despite of that the content of fish meal in fish diets has been reduced, this problem has not been completely eliminated. It is because diets without fishmeal have lower palatability and consequently farmed fish frequently show reduced growth.
By reducing fishing and increase demand for cultured products, the price of fishmeal has been increased continuously and, subsequently, the price of fish feed also increased (Tacon and

Metian, 2008). So, feeding is currently one of the most expensive factors in aquaculture production (FAO, 2006).

Compound feeds for herbivorous-omnivorous fish, such as tilapias, often contain about 15% fishmeal, whereas a typical diet for carnivorous fish, such as farmed salmon, contains 20-30% fishmeal and 15-20% fish oil. Similarly, compound commercial shrimp feeds contain about 25% fish meal (Tacon and Barg, 1998). Additionally, aquafeed manufacturers often over-formulate feeds for other fish species, i. e. the fish meal content. This excessive use of fish meal occurs in part because dietary information for particular species is insufficient. Thus, in addition to the need for replacement of fishmeal in diets for fish, it is also necessary to have detailed information about the nutritional requirements of farmed fish species.

-Why was included fish meal at a level of 3%?

The function and effect of fishmeal as feed for fish is expected only when it is used in high inclusion levels. The purpose of using 3% fishmeal in the present study was only intended as a way to increase the palatability of the tested diets and maintain a homogeneous consumption of the feed offered (ad libitum) during the experimental periods. In **Chapter 3** feed consumption during the experimental period ($g.fish^{-1}$) was homogeneous for *P. brachypomus* and ranged from 11.5 (control diet) to about 13.0 (WD and WF diets at 15 and 25%). In **Chapter 4**, contrary to this phenomenon, the feed consumption by *O. niloticus* increased from 21.9 to 30.5 $g.fish^{-1}$ with the increasing inclusion level of aquatic plants in the diet, particularly for the WF25 diets. The experimental diets were not rejected by fish, but the increase in feed consumption can be explained by the decrease in the digestibility of diets with a higher content of aquatic plants.

-Why was wheat bran replaced?

Wheat bran was replaced by fermented duckweeds (*Lemna minor* and *Spirodela polyrhiza*) and by fermented water fern (*Azolla filiculoides*) at 15% and 25% due to its comparable nutrient composition with the aquatic plants. In this way it was possible to maintain the content of protein (isonitrogenous) and energy (isoenergetic) of the low-fishmeal diets. A similar procedure was reported by El-Sayed (2003) who also replaced wheat bran by fresh and fermented aquatic plants at 10 and 20% in experiments with Nile tilapia.

Growth Trials

The results in **Chapter 3 and 4** showed that the inclusion of fermented aquatic plants up to 15% in low-fishmeal diets affected positively the growth parameters of the species *Piaractus brachypomus* but do not show significantly differences for the species *Oreochromis niloticus*. This indicates that the offered diets influenced differently the physiological response of these species.
The specific growth ratio (SGR) ($\%.d^{-1}$) for *O. niloticus* varied between 2.8 (control diet, WF25) and 2.9 (DW15, DW25 and WF15) without differences. Contrary, the inclusion of 15% of aquatic plants in diets for the species *P. brachypomus* results in better SGR (3.6) in comparison to the control diet (3.3) and in the 25% group (3.2). Although *O. niloticus* did not show significant differences on growth, a slightly better response was observed in fish which were fed on duckweeds, whereas *P. brachypomus* responded most positively to diets with a maximum inclusion of 15% of both aquatic plants.
Due to their habits primarily herbivores, *O. niloticus* responds well to diets with low fish meal content and high plant protein content. The inclusion of fermented aquatic plants (duckweeds up to 25% and water fern up to 15%) did not affect the growth of *O. niloticus*, but feed efficiency was negatively affected, especially for fish fed WF25 diets.

For *P. brachypomus* whose eating habits are primarily omnivorous, the inclusion of the fermented aquatic plants instead affected positively the growth and feed efficiency of fish which were fed on the diets of the 15% group. The good amino acid profile of the aquatic plants suggests a better balance of amino acids in the low-fishmeal diet and therefore a higher availability of the essential nutrients. Differences in dry matter digestibility of diets may explain this difference between the two fish species. While *P. brachypomus* fed on the control diet and the 15% group presented ADC of the dry matter of 90%, the ADC for *O. niloticus* was about 88% and 79% in the control diet and in the 15% group, respectively.
A higher difference in the ADC was observed by the group of diets with an inclusion level of aquatic plants of 25% for both species of fish. *P. brachypomus* fed DW25 and WF25 showed ADC of 69% and 75%, respectively, while Tilapia showed ADC of 58% and 50% respectively. The ADC of dry matter for the 25% group was about 10% (DW) and 25% (WF) higher for the *P. brachypomus* compared with Nile tilapia.

In **Chapter 5**, the productive performance parameters of the species *P. brachypomus* and *O. niloticus* in a polyculture revealed no significant differences in the growth response with the inclusion of fermented duckweeds at 15% into a commercial diet. This might signify an important reduction of the feeding cost by the substitution of 15% of commercial feed, even if the production costs were not estimated in this work.

After a experimental period of 120 days, the highest weight gain (WG) was obtained for fish fed on DW15 resulting in 444 g and 176 g for *P. brachypomus* and *O. niloticus*, respectively. Fish fed on commercial diet showed a WG of 414 g for *P. brachypomus* and 166 g for *O. niloticus*. No significant differences were observed between the control diet and the DW15 diet. Fish fed on WF15 showed the lowest WG of 382 g (*P. brachypomus*) and of 167 g (*O. niloticus*). A feeding exclusively based on aquatic plants is not recommendable; but their fermentation and combination with other locally available by-products of agriculture or even with commercial feeds might considerably reduce the feeding cost in the rural aquaculture.

Implications for the Rural Aquaculture

The term "rural" describes areas with relatively low population density and whose economy depends highly on agriculture. Thus, rural fish farming refers to a productive activity practiced by farmer families for domestic consumption or partial marketing (Edwards and Demaine, 1997). Rural aquaculture is a profitable activity, which can lead to social, economic and ecological benefits, especially when it is integrated with other agricultural activities. The enabling environment for the development of rural aquaculture comprises several factors: the need for governmental support to the planning and teaching, facilities to promote the growth of aquaculture, access to quality inputs such as fingerlings, low-cost feed (as alternative to fishmeal), financial services and access to market. All these factors muss be taken into account to establish successfully the rural aquaculture. In Asian and Africa several researches have been developed works in this field (El-Sayed, 2008; Kalita, P. et al., 2007, Yu, Y., 2004; El-Sayed, 2003; Tacon, 1993; Tacon, 1994; Ray and Das, 1995). However the majority of these results have a local application. For South America there are very few studies related to these topics. In Latin America, aquaculture is mainly focused on intensive export oriented production and therefore it is highly depending on imports of fish feed or pelleted diets (Flores-Nava, A. 2007).

Not all the above mentioned aspects are part of the present study, but it is necessary to point them out due to the complexity of the aquaculture sector. To contribute to the establishment of successful rural aquaculture, this work was focused on the basic aspects related to low cost available feed alternatives, native cultured fish and extensive polyculture.

-Why is the cultivation of native fish species preferable?

The main reason for the use native species in rural aquaculture is that they are adequately suited to the characteristics of the local aquatic environment. Otherwise, the introduction of exotic species represents a threat to the native wildlife and the ecological balance of the ecosystems where aquaculture is developed.

-Why herbivorous/omnivorous fish?

Formulated feeds for herbivorous and omnivorous fish frequently contain lower levels of protein from fish and other terrestrial animals than compound feeds for carnivorous cultured fish, whose dominant ingredients are fish meal and fish oil. This occurs because plant-based proteins and oils are often deficient in essential amino acids (such as lysine and methionine) and fatty acids as eicosapentanoic acid (EPA) and docosahexanoic acid (DHA). These two ingredients are important because they provide energy, and fish convert energy more inefficiently from proteins and oils than from carbohydrates.

The required dietary protein per unit weight for farmed herbivorous, omnivorous and carnivorous fish is very similar. But herbivorous and omnivorous freshwater cultured fish utilize plant-based proteins and oils better than carnivorous fish, so they require lower quantities of fish meal to supply essential amino acids (Naylor et al. 2001).

-Why extensive polyculture?

Intensive systems for herbivorous and omnivorous fish rely heavily on added feeds, because fish are stocked at high densities that cannot be supported by natural food sources. Besides, the use of extensive or semi-intensive culture systems usually involves less sophisticated methods, depends on natural food and is less expensive than intensive systems (FAO, 2002). Polyculture, in turn, maximizes the production through the cultivation of an appropriate combination of fish species with different feeding habits, allowing better use of the natural food available in the pond (De la Lanza-Espino et al., 1991).

In general, the growth performance of *P. brachypomus* fed the selected aquatic plants was beyond the expected, being this the first time that results on the effect of using aquatic plants in diets for this species are reported. *O. niloticus* also responded to the expectations and the obtained results for this fish species were consistent with those reported in the literature.
The locally available aquatic plants have clearly a great potential as feed ingredient for fish diets in rural zones of Colombia. Fermentation of the plant material is highly recommended to conserve their nutritional characteristics and to increase their digestibility by fish. Sun drying of aquatic plants is not recommended, neither their exclusive use as feed for Nile tilapia or Cachama blanca. The inclusion of duckweeds and water fern up to 15% in formulated low-fish meal content diets affects positively fish growth of Cachama; the inclusion of the selected aquatic plants above this level reduces the fish growth significantly. Nile tilapia responds well to low-fish meal content diets. The inclusion of duckweeds and water fern up to 25% in the formulated diets does not influence growth, but the feeding efficiency is negatively affected, especially if diets include water fern at 25%.
Replacement of 15% of a commercial diet (24 % crude protein, 30% fish meal) by the fermented duckweeds did not significantly affect the growth response of Nile tilapia and Cachama blanca in polyculture. This is perhaps the most important result of this study, as this may mean that the feeding cost in the rural aquaculture might be considerably reduced by the use of these available aquatic plants and provide to the small-scale farmers the opportunity to compete in local markets.

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Tables

Figures

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I hereby confirm that this dissertation entitled "The Use of Locally Available Aquatic Macrophytes as Fish Feed for Rural Aquaculture Purposes in South America" does not contain without acknowledgement any material previously submitted for a degree or diploma in any university and that to my knowledge, it does not contain any material previously released or written by another person where due reference is not met in the text.

I have been working on my thesis with full knowledge of the Ph.D. regulations of the Faculty of Agriculture and Horticulture of Berlin dated 14^{th} July 2005.

Hiermit versichere ich, die vorliegende Arbeit ausschließlich auf Grundlage der angegebenen Hilfsmittel und selbständig verfasst zu haben.

Zudem wurde die vorliegende Arbeit in keinem anderen Promotionsverfahren angenommen oder abgelehnt.

Ich habe die Arbeit in Kenntnis der Promotionsordnung vom 14.07.2005 der Landwirtschaftlich-Gärtnerischen Fakultät zu Berlin angefertigt.